Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L4	4	(("5541161") or ("4530836")).PN.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/12/15 08:57
L5	106	(514/487).CCLS.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/12/15 08:57
L6	710	(560/13).CCLS.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/12/15 08:58

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NEWS 1   Web Page URLs for STN Seminar Schedule - N. America
NEWS 2   "Ask CAS" for self-help around the clock
NEWS 3   SEP 09   ACD predicted properties enhanced in REGISTRY/ZREGISTRY
NEWS 4   OCT 03   MATHDI removed from STN
NEWS 5   OCT 04   CA/CAplus-Canadian Intellectual Property Office (CIPO) added
                   to core patent offices
NEWS 6   OCT 13   New CAS Information Use Policies Effective October 17, 2005
NEWS 7   OCT 17   STN(R) AnaVist(TM), Version 1.01, allows the export/download
                   of CAplus documents for use in third-party analysis and
                   visualization tools
NEWS 8   OCT 27   Free KWIC format extended in full-text databases
NEWS 9   OCT 27   DIOGENES content streamlined
NEWS 10   OCT 27   EPFULL enhanced with additional content
NEWS 11   NOV 14   CA/CAplus - Expanded coverage of German academic research
NEWS 12   NOV 30   REGISTRY/ZREGISTRY on STN(R) enhanced with experimental
                   spectral property data
NEWS 13   DEC 05   CASREACT(R) - Over 10 million reactions available
NEWS 14   DEC 14 2006 MeSH terms loaded in MEDLINE/LMEDLINE
NEWS 15   DEC 14 2006 MeSH terms loaded for MEDLINE file segment of TOXCENTER
NEWS 16   DEC 14 CA/CAplus to be enhanced with updated IPC codes

NEWS EXPRESS   DECEMBER 02 CURRENT VERSION FOR WINDOWS IS V8.01,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 02 DECEMBER 2005.
               V8.0 USERS CAN OBTAIN THE UPGRADE TO V8.01 AT
               http://download.cas.org/express/v8.0-Discover/

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FILE 'HOME' ENTERED AT 12:43:12 ON 15 DEC 2005

=> fil reg
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                                              ENTRY   SESSION

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DICTIONARY FILE UPDATES: 14 DEC 2005   HIGHEST RN 869939-98-0

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****
*   *
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added,   *
* effective March 20, 2005. A new display format, IDERL, is now   *
* available and contains the CA role and document type information. *
*   *
**

Structure search iteration limits have been increased. See HELP SLIMITS
for details.

REGISTRY includes numerically searchable data for experimental and
predicted properties as well as tags indicating availability of
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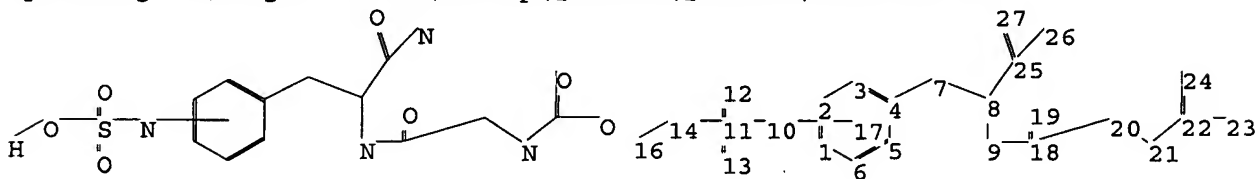
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ring nodes :
1 2 3 4 5 6
chain bonds :
4-7 7-8 8-9 8-25 9-18 10-11 11-12 11-13 11-14 14-16 18-19 18-20 20-21
21-22 22-23 22-24 25-26 25-27
ring bonds :
1-2 1-6 2-3 3-4 4-5 5-6
exact/norm bonds :
8-9 9-18 10-11 18-19 20-21 21-22 22-23 22-24 25-26 25-27
exact bonds :
4-7 7-8 8-25 14-16 18-20
normalized bonds :

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isolated ring systems :
containing 1 :

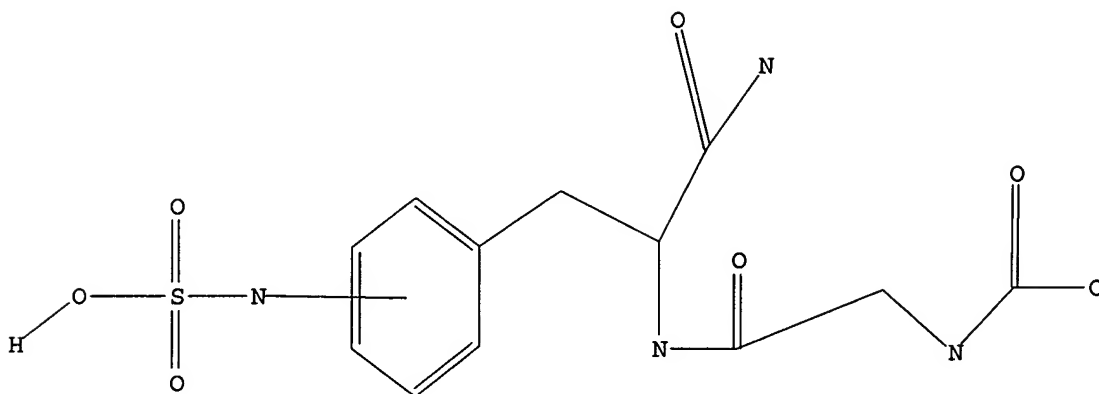
Match level :
1:Atom 2:Atom 3:Atom 4:Atom 5:Atom 6:Atom 7:CLASS 8:CLASS 9:CLASS 10:CLASS
 11:CLASS 12:CLASS 13:CLASS 14:CLASS 16:CLASS 17:CLASS 18:CLASS 19:CLASS
 20:CLASS 21:CLASS 22:CLASS 23:CLASS 24:CLASS 25:CLASS 26:CLASS 27:CLASS

L1   STRUCTURE UPLOADED

=> d
L1 HAS NO ANSWERS
L1   STR

Structure attributes must be viewed using STN Express query preparation.

=> s l1
SAMPLE SEARCH INITIATED 12:43:33 FILE 'REGISTRY'
SAMPLE SCREEN SEARCH COMPLETED -   6 TO ITERATE

100.0% PROCESSED   6 ITERATIONS   0 ANSWERS
SEARCH TIME: 00.00.01

FULL FILE PROJECTIONS: ONLINE **COMPLETE**
                        BATCH   **COMPLETE**
PROJECTED ITERATIONS: 6 TO   266
PROJECTED ANSWERS: 0 TO   0

L2   0 SEA SSS SAM L1

=> s l1 full
FULL SEARCH INITIATED 12:43:37 FILE 'REGISTRY'
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100.0% PROCESSED   104 ITERATIONS   17 ANSWERS
SEARCH TIME: 00.00.01

L3   17 SEA SSS FUL L1

=> s l3 and caplus/lc
    49173719 CAPLUS/LC
L4   17 L3 AND CAPLUS/LC

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=> fil caplus
COST IN U.S. DOLLARS                         SINCE FILE      TOTAL
                                                ENTRY       SESSION
FULL ESTIMATED COST                            165.93       166.14


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FILE LAST UPDATED: 14 Dec 2005   (20051214/ED)

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=> s l4
L5              3 L4

=> d ibib abs hitstr 1-3
```

```
ACCESSION NUMBER:        2004:701811 CAPLUS
DOCUMENT NUMBER:         141:225833
TITLE:                   Preparation of (sulfonylamino)phenylalaninamide
                         derivatives and related compounds
INVENTOR(S):             Klopfenstein, Sean Rees; Maier, Matthew Brian; Jones,
                         David Robert; Gray, Jeffrey Lyle; Pokross, Matthew
                         Eugene; Peters, Kevin Gene; Evdokimov, Artem Gennady
PATENT ASSIGNEE(S):      The Procter & Gamble Company, USA
SOURCE:                  U.S. Pat. Appl. Publ., 41 pp.
                         CODEN: USXXCO
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  1
PATENT INFORMATION:

        PATENT NO.          KIND    DATE       APPLICATION NO.        DATE
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        US 2004167183       A1      20040826   US 2003-633743        20030804
        US 2005154011       A1      20050714   US 2003-633744        20030804
        WO 2004074238       A1      20040902   WO 2003-US26596       20030825
            W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
                CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
                GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
                LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
                PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN,
                TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
            RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
                KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
                FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
                BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
        WO 2004074256       A1      20040902   WO 2003-US26598       20030825
            W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
                CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
                GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
                LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, OM,
                PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN,
                TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
            RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
                KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
                FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
                BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.:                         US 2003-448749P     P  20030220

                                               US 2003-455977P     P  20030318

OTHER SOURCE(S):         MARPAT 141:225833
GI
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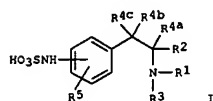

AB   The invention relates to compds. I [R1 is -L1-(CR6aR6b)0-5-R7, where L1
is

```
a bond, O, S, N, CO2, SO2, CSNR8, CONR8O, etc, where R8 is H or
(un)substituted alkyl; R6a, R6b are H, R9, OR9, etc., where R9 is H,
(un)substituted alkyl, aryl or alkylenearyl or two R9 units or R7/R9 can
form a ring; R7 is nil, H, (un)substituted alkyl, hydrocarbyl,
heteroaryl,
etc.; R2 is -(CH2)0-5-L2-(CR11aR11b)0-5-R12, where L2, R11a/R11b and R12
are groups similar to L1, R6a/R6b and R7, resp.; R3 is -(CH2)0-5-L3-R10,
where L3 is a group similar to L1 and R10 is H, (un)substituted alkyl,
heteroalkyl, aryl, etc.; R4a, R4b, R4c, and R5 are H or a substituent; or
R2 and R4a, R4a and R4b, R1 and R2, or R1 and R3 can form a carbocyclic
or
heterocyclic ring] which are effective in the treatment of protein
tyrosine phosphatase (PTPase) mediated disorders such as diabetes.  Thus,
Boc-D-Phe(NHSO3H-4)-NHMe (Boc = tert-butoxycarbonyl) was prepd. from
Boc-D-Phe(NO2-4)-OH by amidation with methylamine, catalytic
hydrogenation, and reaction with sulfur trioxide-pyridine complex.
IT    680576-86-7P 746674-61-3P 746674-62-4P
      746674-63-5P 746674-64-6P 746674-65-7P
      746674-66-8P 746674-67-9P 746674-69-1P
      746674-32-4P 746676-33-5P 746676-34-6P
      746676-35-7P 746676-36-8P 746676-37-9P
      746676-38-0P
RL:  PAC (Pharmacological activity); SPN (Synthetic preparation); THU
     (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES
     (Uses)
     (preparation of (sulfonylamino)phenylalaninamide derivs. and related
     compds.)
RN   680576-86-7  CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-tyrosyl-N-methyl-4-
     (sulfoamino)- (9CI)  (CA INDEX NAME)
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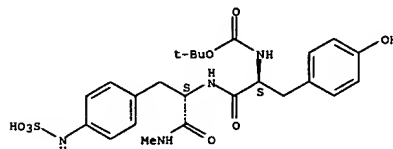
Absolute stereochemistry.


```
RN   746674-61-3  CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-leucyl-N-methyl-4-
     (sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
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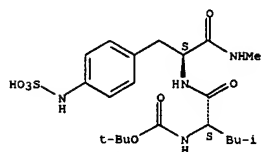
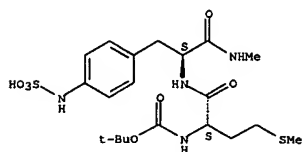Absolute stereochemistry.

● NH3

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RN   746674-62-4  CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-methionyl-N-methyl-
     4-(sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
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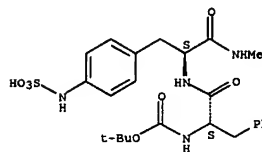Absolute stereochemistry.

● NH3

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RN   746674-63-5  CAPLUS
CN   L-Phenylalaninamide, N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl-N-
     methyl-4-(sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
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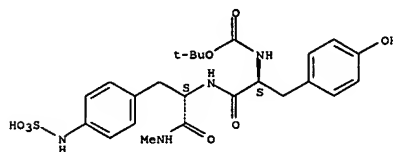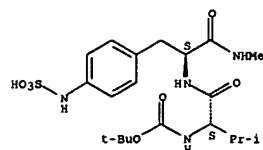Absolute stereochemistry.

● NH3

```
RN   746674-64-6  CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-tyrosyl-N-methyl-4-
     (sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
```

Absolute stereochemistry.

● NH3

```
RN   746674-65-7  CAPLUS
CN   L-Phenylalaninamide, N-[(1,1-dimethylethoxy)carbonyl]-L-valyl-N-methyl-4-
     (sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
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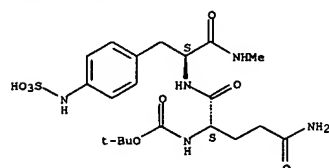
Absolute stereochemistry.

● NH₃

RN   746674-66-8 CAPLUS
CN   L-Phenylalaninamide, N2-[(1,1-dimethylethoxy)carbonyl]-L-glutaminyl-N-
      methyl-4-(sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)
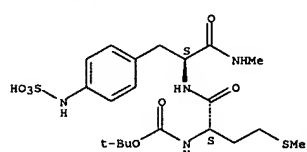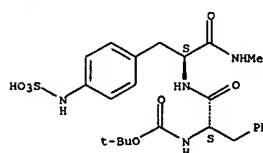
Absolute stereochemistry.

● NH₃

RN   746674-67-9 CAPLUS
CN   L-Phenylalaninamide, N2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-N-
      methyl-4-(sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

● NH₃

RN   746674-69-1 CAPLUS
CN   L-Phenylalaninamide, N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl-N-
      pentyl-4-(sulfoamino)-, monoammonium salt (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

● NH₃

RN   746676-32-4 CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-leucyl-N-methyl-4-
      (sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

RN   746676-33-5 CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-methionyl-N-methyl-
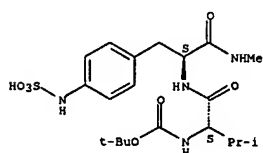   4-(sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

RN   746676-34-6 CAPLUS
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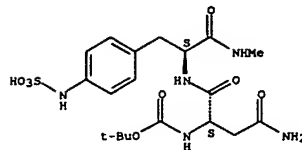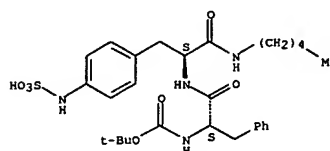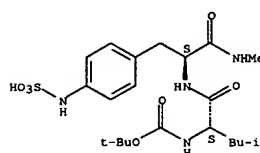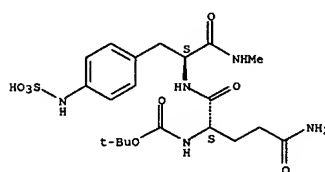   methyl-4-(sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

RN   746676-35-7 CAPLUS
CN   L-Phenylalaninamide, N-[(1,1-dimethylethoxy)carbonyl]-L-valyl-N-methyl-4-
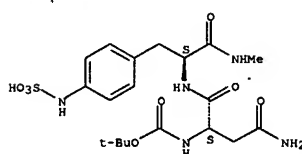   (sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

RN   746676-36-8 CAPLUS

CN   L-Phenylalaninamide, N2-[(1,1-dimethylethoxy)carbonyl]-L-glutaminyl-N-
      methyl-4-(sulfoamino)- (9CI)   (CA INDEX NAME)

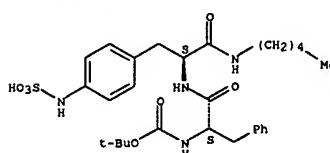Absolute stereochemistry.

RN   746676-37-9 CAPLUS
CN   L-Phenylalaninamide, N2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-N-
      methyl-4-(sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

RN   746676-38-0 CAPLUS
CN   L-Phenylalaninamide, N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl-N-
      pentyl-4-(sulfoamino)- (9CI)   (CA INDEX NAME)

Absolute stereochemistry.

ACCESSION NUMBER: 2004:331682 CAPLUS
DOCUMENT NUMBER: 140:352651
TITLE: The three-dimensional structure of protein tyrosine
                         phosphatase β subunit and its use in drug design
INVENTOR(S):   Evdokimov, Artem Gennady; Pokross, Matthew Eugene
PATENT ASSIGNEE(S):   The Procter & Gamble Company, USA
SOURCE: U.S. Pat. Appl. Publ., 335 pp.
                         CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004077065	A1	20040422	US 2003-634027	20030804
PRIORITY APPLN. INFO.:			US 2002-413547P P	20020925

AB   The crystal structures of the catalytic domain of the human protein
      tyrosine phosphatase HPTPβ, in ligand-bound and ligand-free forms are
      described. These structures are useful in computer aided drug design for
      identifying compds. that bind or activate HPTPbeta and thereby modulate
      angiogenesis mediated disorders or diseases.
IT   680576-86-7P
      RL: BSU (Biological study, unclassified); SPN (Synthetic preparation);
      BIOL (Biological study); PREP (Preparation)
          (as inhibitor of HPTPβ, design and synthesis of; three-dimensional
          structure of protein tyrosine phosphatase β subunit and its use in
          drug design)
RN   680576-86-7 CAPLUS
CN   L-Phenylalaninamide,
N-[(1,1-dimethylethoxy)carbonyl]-L-tyrosyl-N-methyl-4-
      (sulfoamino)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.
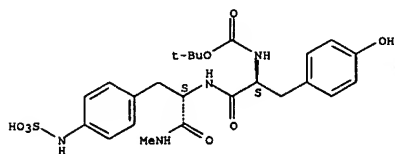
ACCESSION NUMBER: 1989:1067 CAPLUS
DOCUMENT NUMBER: 110:1067
TITLE: Structure-activity relationship studies on
                         cholecystokinin: analogs with partial agonist
                         activity
AUTHOR(S):   Galas, Marie Christine; Lignon, Marie Francoise;
                         Rodriguez, Marc; Mendre, Christiane; Fulcrand,
Pierre;
                         Laur, Jeanine; Martinez, Jean
CORPORATE SOURCE: Cent. Pharmacol.-Endocrinol., Montpellier, 34094, Fr.
SOURCE: American Journal of Physiology (1988), 254(2, Pt. 1),
                         G176-G182
                         CODEN: AJPHAP; ISSN: 0002-9513
DOCUMENT TYPE: Journal
LANGUAGE: English
AB   Hepta- and octapeptide analogs of the C-terminal part of cholecystokinin
      (CCK), modified on the C-terminal phenylalanine residue, were studied.
In
      these CCK analogs, the peptide bond between aspartic acid and
      phenylalanine had or had not been modified and they were lacking the
      C-terminal primary amide function. These CCK derivs. were able to cause
      full stimulation of amylase release from rat pancreatic acini but without
      a decrease in amylase release at supramaximal concns. There was a close
      relation between the abilities of these derivs. to inhibit binding of
      release and their abilities to inhibit binding of [125I]Bolton-Hunter-
      labeled CCK-9 to CCK receptors on rat and guinea pig pancreatic acini.
      These CCK analogs were also able to recognize the guinea pig brain CCK
      receptors, some of them being particularly potent. The aromatic ring of
      phenylalanine was important for the binding to brain and pancreatic CCK
      receptors, whereas the C-terminal primary amide function was not
essential
      for the binding to pancreatic CCK receptors but was crucial for complete
      biol. activity toward rat pancreatic acini.
IT   117829-66-0
      RL: BIOL (Biological study)
          (pancreas binding and biol. activity of, structure in relation to)
RN   117829-66-0 CAPLUS
CN   L-α-Asparagine, N2-[N-[N-[N-[N-[N-[N-[(1,1-dimethylethoxy)carbonyl]-
      L-α-aspartyl]-4-(sulfoamino)-L-phenylalanyl]-L-norleucyl]glycyl]-L-
      tryptophyl]-L-norleucyl]-N-(2-phenylethyl)- (9CI) (CA INDEX NAME)

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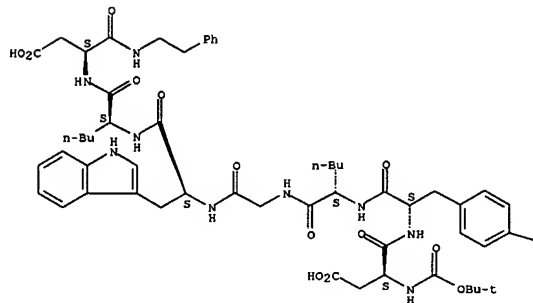
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Recent discovery and development of protein tyrosine phosphatase inhibitors

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The protein tyrosine phosphatases (PTPases or PTPs) play an important role in controlling the status of tyrosine phosphorylation and the regulation of cellular function. The ability to selectively inhibit PTPs holds enormous therapeutic potential for the treatment of diseases such as diabetes, cancer and osteoporosis. However, an understanding of the role of many PTPs has yet to be unravelled, with only PTP1B convincingly validated as a therapeutic target. Furthermore, the intricate network of different PTPs extensively involved in signalling events requires high selectivity for the desired PTP target, in order to minimise potential side effects. Most research programmes into PTP inhibitors are still at an early stage and have yet to convert initial leads into compounds with more drug-like properties. Inhibitors have been identified by modification of peptide substrates, from natural product screening and by rational design. This article will give an overview of PTPs, followed by a more detailed description of the development of PTP inhibitors. Patents on PTP inhibitors published between January 1998 and Feb 2002 will be discussed in the context of the available literature.

1. Introduction

Protein phosphorylation is a fundamental component of biological signalling processes, acting to regulate the activity of proteins via modification of amino acid side chains. The phosphorylation of proteins is catalysed by the protein kinase class of enzymes, while dephosphorylation is regulated by protein phosphatases. The protein phosphatases can be separated into different classes based upon the substrate specificity of the enzymes; protein phosphatases (PPs or PSPs) hydrolyse phosphoester side chains on serine or threonine residues, while protein tyrosine phosphatases (PTPs) are specific for phosphorylated tyrosine side chains. The PTP class of enzymes also includes a subfamily of dual specificity PTPs (DSPs), which are capable of dephosphorylating both phosphotyrosine and phosphoserine/phosphothreonine [1]. This review will focus on inhibition of the PTPs, including DSPs, and resulting therapeutic applications.

1.1 Biological properties of PTPs
The PTPs are critical components of signal transduction pathways, with the regulation of tyrosine phosphorylation playing a role in cell growth, adhesion, metabolism and differentiation. A number of reviews on signalling [19] and the role of PTPs in signalling have appeared in the past decade [2-11]. The August 2001 issue of *Chemical Reviews* [12] was devoted to protein phosphorylation and signalling, including articles on the structural basis for control by phosphorylation [13], a phylogenetic overview of the protein phosphatases [14], a discussion of the structure and catalytic mechanisms of the protein phosphatases [15], regulatory mechanisms

for PTPs [15,16], the involvement of non-receptor PTPs in MAPK cellular signalling [17] and potential roles of DSPs [18]. The recognition that tyrosine phosphorylation is a major mechanism of transmembrane and intracellular signalling has gradually developed since the mid 1980's [19], with the first phosphatases isolated in the early 1980s [3]. For example, one of the most important PTPs for therapeutic use, PTP1B, was only isolated (from human placenta) and characterised in 1988 [20,21]. Elucidation of the role of PTPs has been even more delayed; protein phosphatases were initially 'believed to be few in number and consigned to boring housekeeping roles' [22] by simply dephosphorylating products of protein kinase activity, in other words, they were 'regarded as constitutively activated enzymes that reversed the activity of inducibly activated protein kinases' [19]. The identification of mechanisms controlling activation/deactivation and specificity of PTPs, such as phosphorylation, subcellular localisation or dimerisation, is ongoing [19,22].

Over 75 PTPs, with a variety of structures and functions, have been identified [2]. An initial analysis of the newly sequenced human genome by the International Human Genome Sequencing Consortium indicates 112 potential human PTPs/DSPs [23], while the Celera-led sequencing effort predicts 56 PTPs and 29 DSPs [24]. PTPs consist of a conserved phosphatase domain in conjunction with a variety of other domains. Classical non-transmembrane (soluble) PTPs, which include PTP1B, TCPTP, SHP-1 and SHP-2, are comprised primarily of the PTP domain. A subclass of the soluble PTPs are low molecular weight PTPs, with M_r near 18,000, compared with 30,000 – 50,000 for the classical soluble PTPs [25]. Receptor-PTPs (RPTPs) are much more complex and contain a transmembrane domain, one or two intracellular phosphatase domains and an extracellular segment [2]. In most cases of RPTPs with two PTP domains, the C-terminal PTP domain has little or no catalytic activity [22]. RPTPs include PAR, CD45 and PTP-α, β, γ, δ, ϵ, λ, κ, μ, σ and ζ. The PTP subfamily of DSPs, which includes subgroups of VHR-like, Cdc25-like and MAPK phosphatases (MKPs), has a PTP domain with reduced homology to the PTP domain of classical PTPs. A schematic representation of the tyrosine phosphatase superfamily, illustrating the various non-catalytic domains associated with many of the PTPs, has been published [22].

The extracellular domain of many RPTPs appears to play a role in cell adhesion [26]. For example, PTP-ζ/β may play a role in neuronal cell adhesion, with the extracellular segment binding contactin (a neuronal cell recognition molecule) [1,27]. Other RPTPs, such as PTP-μ [28] and PTP-κ [29], have extracellular domains that associate with and may regulate, cadherin complexes, which are involved in cell–cell adhesion. Disruption of adhesion could result in metastasis [2]. Other PTPs (SHP-1, SHP-2, Csw) contain SH2 domains, that recognise specific phosphorylated tyrosines; phosphorylation/dephosphorylation of the target ligand regulates association with the SH2 domain. Some of these interactions can be

quite complex. A crystal structure shows that the N-terminal SH2 domain of SHP-2 PTP blocks substrate access to the catalytic site by binding to the catalytic cleft. Binding of a phosphotyrosine-containing ligand to the SH2 domain unblocks the active site, activating the enzyme [30,31].

Substrates of PTPs have been identified by using substrate-trapping mutants in which either the active site catalytic nucleophilic Cys residue is mutated to Ser (PTP1B C215S mutant [32-34]; PTP-PEST C231S mutant [34,35]; SHP-1 C455S mutant [36]) or the catalytic general acid Asp residue is changed to an Ala (PTP1B D181A mutant [34,37,38]; PTP PEST D199A mutant [34]; cyt-PTP-ϵ D245A mutant [39]) or Asn residue (PTP1 D181N or VHR D92N mutants [40]). The mutants lose much or all of their catalytic capabilities as their ability to dephosphorylate the pTyr residue is either impaired (Asp mutants) or abrogated (Cys mutants). However, the mutants still retain high affinity for their substrates, allowing the most potent targets to be trapped from a mixture of potential substrates. For example, a PTP-PEST D199A mutant was used to identify p130^{cas} as a specific substrate via its exclusive selection from a mixture of 50 – 100 pTyr-containing proteins that were incubated with the mutant bound to an affinity matrix [34]. The Asp mutants appear to exhibit better substrate-trapping properties than the Cys mutants, possibly because the substrate can still form a covalent bond between the active-site site and the pTyr residue [34] and the potential electrostatic repulsion between the PTP Asp carboxylate and the substrate phosphonate groups is removed [22].

One potential drawback to the substrate trapping approach is that by altering the active site residues, substrate specificity may be affected by perturbations in the active site. Some active site differences have been observed for the C215S mutant [41]. Another limitation is that this method has generally identified only a few, mostly abundant, phosphoproteins. The mutants may have insufficient affinity to identify less common substrates [41]. Therefore, a double D181A/Q262A PTP1B mutant has been prepared, possessing 6-fold higher affinity than the D181A mutant and 28-fold higher affinity than the C215S mutant [41]. The additional mutated Gln262 is a conserved residue that is normally used to increase the rate of hydrolysis of the thiophosphoryl-enzyme intermediate. The double mutation further reduces the residual phosphatase activity of the D181A general base mutant and results in higher substrate affinity than C215S/Q262A, D181A/C215S or D181A/C215E double mutants [41]. When expressed in COS1 cells, the D181A/Q262A mutant trapped several new pTyr proteins, other than the abundant phosphorylated EGF receptor, which is the only phosphoprotein trapped by the D181A or C215S mutants [41].

Other uses for PTP-trapping mutants have been identified. A PTP1B D181A substrate-trapping mutant has been expressed in a mouse fibroblast cell and used to demonstrated that, after internalisation of activated receptor tyrosine kinases from the cell surface, the receptors are transported to the endoplasmic reticulum (ER), where PTP1B dephosphorylates the

cytoplasmic domain pTyr residues [42,43]. Substrate trapping PTPs have been patented [301], as has their use in an assay method [302]. A patent claiming PTP1B and PTP-PEST mutants states that if this mutant protein is administered to a human it would be able to trap the substrate and inhibit the signalling pathway, thus being potentially useful as a treatment for cancer [301]. Substrate-trapping mutants may exist in nature. A naturally-occurring catalytically inactive DSP (no active site Cys), which could bind to phosphoproteins and protect them from dephosphorylation, has potentially been identified [18,44].

The specificity of PTPs and evidence for how this is achieved, was reviewed in 2001 [22]. PTPs were initially believed to have little substrate specificity, with intracellular targeting allowing for selective dephosphorylation. Some of the PTP domains are believed to play a role in directing the PTPs to their correct location [8]. However, more recent research indicates that a high degree of specificity is achieved by various combinations of spatial targeting strategies and intrinsic catalytic domain substrate specificity [22]. For example, the classical non-transmembrane PTPs PTP1B and TCPTP possess ~ 75% sequence identity in their catalytic domains; substrate-trapping mutants demonstrate that they dephosphorylate different sets of target substrates. Further specificity is provided by targeting domains that localise the PTPs to different intracellular compartments [22]. Specificity can also be achieved by targeting the substrates to the enzyme. A recent study demonstrated that after internalisation of activated receptor tyrosine kinases (epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor-β (PDGFR)) from the cell surface of a mouse fibroblast cell, they are transported to the ER, where PTP1B dephosphorylates the cytoplasmic domain pTyr residues [42,43]. PTP1B was shown to cluster in specific regions of the ER in response to growth-factor stimulation of the cells. The trapping and localisation were visualised by fluorescence energy transfer (FRET) methods, with the receptors tagged with green fluorescent protein and the PTP1B with sulfoindocyanine-conjugated mAbs.

The elucidation of mechanisms controlling activation/deactivation of PTPs is ongoing [19,22]. Discussions of mechanisms of regulation were included in both a 1998 [45] and a 2000 [46] review of PTPs. A dimerisation mechanism has been proposed as a method of inhibition of RCPTs, such as CD45 and RPTPα [47-50]. As discussed previously, SHP-2 is activated by binding of phosphopeptide/protein ligands to the SH2 domain, altering its conformation and resulting in removal of the SH2 domain from the SHP-2 active site [30,31]. For example, leptin binding to the leptin receptor has been shown to induce binding of SHP-2 to the receptor, resulting in activation of SHP-2 and a decrease in phosphorylation of JAK2 [51]. Binding of phosphopeptides derived from PDGFR and insulin receptor substrate-1 also activate SHP-2 [30,31]. Another possible mechanism of regulation of tyrosine phosphatases is via oxidative inactivation of the essential active site cysteine. Transient formation of hydrogen peroxide near the active site can induce oxidation of the thiol to give a cysteine–sulfenic acid residue

[15,45,52]. The production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, in cells can be tightly regulated. Further evidence of this mechanism has recently been reported: multiple PTPs were reversibly oxidised and inactivated when Rat-1 cells were treated with hydrogen peroxide [53]. Furthermore, the growth factor PDGF was shown to induce a specific response of transient oxidation/inhibition of SHP-2, as demonstrated by an in-gel PTP activity assay. The oxidation appears to be spatially restricted to SHP-2 that is complexed with PDGFR on the membrane surface [53]. Another possible mechanism to control in vivo phosphorylation is via catalytically inactive PTPs that bind to phosphoproteins and protect them from hydrolysis by other PTPs [18,44].

Thus, a variety of roles have been identified for the non-catalytic domains of PTPs. Given the complexity of biological systems, it is highly likely that more examples of the importance of these regions will emerge in future years, particularly regarding regulation and targeting.

1.2 Structure and mechanism of PTPs

The structure and mechanisms of the protein phosphatases have been reviewed several times [1,4,7,10,15,22,45,54,55]. The two general classes of phosphatases (PPs and PTPs) have distinct structures and mechanisms. The serine/threonine protein phosphatases are a subgroup of a large structural family of metallo-phosphoesterases containing a metal ion centre at the active site (for an X-ray structure see [56]). In contrast, the PTPs do not require metal ions for catalysis. They contain an active site sequence motif of HCXXGXXRS(T) within the catalytic domain [1], which for classical PTPs consists of 240 – 250 conserved residues [22].

Crystal structures of a number of PTPs have been published, including PTP1B with and without bound tungstate [57], with vanadate [58] and with a variety of other inhibitors, such as 2-(difluoromethylenephosphonate)naphthalene [59], 2-(difluoromethylenephosphonate)naphthalene-2-carboxylic acid Glu amide [60], two nonpeptidyl aryl bis(difluoromethylenephosphonate) compounds [61], 2-(oxalyl-amino)benzoic acid (OBA) [62] and OBA analogues [62,63], an O-malonyl-tyrosine-based compound [64], a cyclic peptide containing the pTyr mimetic O-fluoromalonyl-tyrosine [60], a 3′-carboxy-4′-O-(carboxymethyl)tyrosine-based inhibitor [64,65] and a tetrazole analogue [66], a small molecule thiophene-based inhibitor [67] and two benzothiophene biphenyl derivatives [63]. Crystal structures of catalytically inactive PTP1B C215S mutant bound with pTyr [33], with EGFR peptide [32] or with the nonpeptidic substrate bis-(p-phosphophenyl)methane (BPPM) [33] have also been published, as have the structures of catalytically inactive PTP1B C215A mutant bound with phosphopeptides corresponding to the insulin receptor activation loop [38] or a catalytically impaired PTP1B Q262A mutant cysteinyl–phosphate intermediate [58]. The structures of several other PTPs have also been determined, including Yersinia PTP and its complex with tungstate [68,69] or vanadate [40], the catalytic domain of SHP-2 with two SH2 domains [30], the amino terminal SH2 domain of

SHP-2 with bound peptides [31], the catalytic domain of SHP-1 and its complex with tungstate [70], catalytically inactive C455S SHP-1 mutant complexed with two phosphoryl peptides [36], the membrane-proximal catalytic domain of a murine receptor-like PTP (murine RPTP-α) [48], the membrane proximal domain of PTP-μ (RPTP-μ D1) [71], the tandem phosphatase domains of RPTP LAR [72], the catalytic domain of human dual specificity phosphatase Cdc25A [73], Cdc25B catalytic subunit bound to sulfate or tungstate [74], the dual specificity PTP VHR [75], a VHR C124S mutant bound to a bisphosphorylated peptide corresponding to the MAPK activation lip [76] and two reports of a low molecular weight bovine heart PTP [25,77]. An NMR solution structure of low molecular weight bovine heart PTP has also been determined [78]. A table of published crystal structures has been prepared [79].

The overall folding structure of the PTPs and the dual specificity phosphatase VHR are very similar, despite the catalytic domain of the classical PTPs having < 5% sequence identity with the catalytic domain of dual specificity phosphatases. This compares to > 30% sequence identity of the catalytic domain within the classical PTPs [1]. The catalytic domain of the classical PTPs consists of a core of four parallel β-strands surrounded by α-helices. The PTP active site consensus sequence, which has been described in a variety of forms, such as HCXXGXXRS(T) [1], [I/V]HCXAGXXR[S/T]G [2] or (H/V)C(X)$_5$R(S/T) [41], forms a phosphate binding pocket for the substrate and connects a β-strand to an α-helix [1]. Similarly, the low molecular weight PTPs have a four central parallel β-sheet core structure and phosphate binding loop, but the invariant sequence is XCXXXXCRS (actually VCLGNICRS) and the peptide substrate binding region differs significantly [25,77]. The dual specificity PTPs have a conserved HCXXXXXR sequence with a shallower pTyr binding pocket than the classical PTPs [74,75].

The phosphate hydrolysis reaction promoted by PTPs is catalysed by a nucleophilic thiol group on the essential cysteine residue that resides at the base of the active site cleft. Mutation of this cysteine residue to alanine or serine abolishes catalytic activity. The guanidinium group of the conserved ariginine residue near the active site assists in phosphate binding and catalysis. The mechanism of dephosphorylation by PTPs proceeds via initial binding of the dianion of the phosphotyrosine substrate to form an enzyme–substrate (ES) complex. Nucleophilic attack of the active site cysteine thiolate onto the substrate phosphorus atom forms a phosphoenzyme intermediate; the cysteine residue must be deprotonated and an aspartic acid residue protonated for this transfer to occur [1,15,40,80-83]. The active site cysteine residues in PTPs have very low pKa (4.7 – 5.5) compared to typical Cys residues in proteins (pKa = 8.5), allowing for easy formation of the thiolate anion [15]. Two of the substrate non-bridged phosphate oxygens are coordinated by hydrogen bonds to the guanidinium group of the essential arginine residue in the active site loop, with the third oxygen interacting with backbone amide NH groups. When the substrate binds into the active site, a

flexible loop containing the protonated aspartic acid residue (the WPD loop) folds over the active site, allowing the aspartate acid group to act as a general acid and protonate the phenolic oxygen of the tyrosine leaving group. Mutational replacement of the aspartate residue with an asparagine residue greatly reduces the catalytic efficiency [40]. The catalytic transition-state structure has been examined using kinetic isotope effects (for VHR D92N and S131A mutants) [84]. The enzymatic dephosphorylation is completed by hydrolysis of the phosphoenzyme intermediate; the critical aspartate residue may again play a role by acting as a general base to abstract a proton from a water molecule to initiate hydrolysis [1,4]. The conserved serine/threonine residue at the end of the active site consensus sequence is also potentially involved in hydrolysis of the thiophosphate intermediate [15]. An X-ray structure of the cysteinyl–phosphate intermediate in a Q262A mutant has been published [58]. The glutamine normally plays an important role in stabilising/activating a water molecule for hydrolysis of the cysteinyl-phosphate intermediate; by removing its catalytic function the rate of phosphoryl–enzyme hydrolysis is substantially slower than the rate of intermediate formation, allowing the intermediate to accumulate.

In order to design PTP inhibitors with high specificity and selectivity, it is important to identify structural features that would allow differential substrate binding between different PTPs. It has been shown that a loop sequence in PTP1B (and related PTPs), YRDV(46-49), is in contact with the substrate peptide backbone and tyrosine residue and helps to form a deep active site pocket, requiring a long phosphotyrosine residue to reach the catalytic thiol [32]. The dual specificity VHR lacks this extended loop and forms a more shallow pocket ~ 6 Å deep instead of 9 Å. This allows for shorter phosphoserine/threonine side chains to make contact with the catalytic cysteine, accounting for the difference in its substrate preference [15,22]. Crystal structures of PTP1B also provide indications for how it may be possible to obtain specificity for inhibition of PTP1B over other PTPs. Structures of the C215S catalytically inactive mutant with bound pTyr or BPPM showed that the phosphophenol moieties can bind at two mutually exclusive sites [33]. Subsequently, a structure of the PTP1B C215A mutant bound to phosphorylated peptides corresponding to the IR activation loop showed interactions with residues on both sides of the dephosphorylation site of IR pTyr1162. Of particular interest is a special shallow groove that accommodates a second substrate pTyr residue (IR pTyr1163) by forming salt bridges between the phosphate group and two PTP1B arginine residues [38]. Peptides containing the tandem pTyr1162-pTyr1163 sequence found in the IR loop have a 70-fold higher affinity for the D181A PTP1B substrate trapping mutant than comparable mono-pTyr peptides [38]. The preference of PTP1B for substrates with acidic residues on the N-terminal side of the pTyr residue is illustrated by a PTP1B arginine residue interacting with Asp1161 in the IR loop peptide. A comparison of the structure and sequence of PTP1B with those of other PTPs indicates that the binding

site for the second pTyr residue is unique to PTP1B and TCPTP [38]. A group from Pharmacia has published an analysis of *in vitro* interactions of PTP1B with insulin receptors, investigating binding under a variety of conditions, in the presence or absence of phosphopeptides modelled after IR pTyr domains, and with a selective and potent PTP1B inhibitor [85].

Crystal structures of SHP-1 complexed with two phosphoryl peptides have also been reported and used to propose reasons for substrate specificity [36], although questions have been raised about whether the structures represent properly bound catalytic intermediates [22]. VHR differs from other DSPs in that it has a preference for dephosphorylation of pTyr over pThr. A recent crystal structure of C124S catalytically inactive VHR mutant with bound bisphosphorylated peptide corresponding to the MAPK activation loop illustrates why VHR has a preference for hydrolysing pTyr rather than pThr residues within substrates containing a -pThr-Xaa-pTyr- sequence [76]. The pThr residue binds into a basic pocket containing Arg158, while the pTyr binds to the deep active site cleft. The crystal structure provides evidence that the preference for pTyr hydrolysis is due to steric factors in the active site preventing the pThr residue from binding [76]. Indeed, preparation of a double mutant by removing two of the VHR residues believed to cause the steric blocking resulted in an enzyme that hydrolysed pThr in a peptide with 9-fold higher activity than native VHR [76].

1.3 Role of PTPs in disease and potential for therapeutic application

Several summaries of the roles of PTPs in diseases have been published [9,22,46,79,86-88], including a 1997 lecture on 'The phosphorylation of proteins on tyrosine: its role in cell growth and disease' [9].

The large number of PTPs, the nascent stage of their discovery and research into their biological role and the many abnormalities associated with some PTP knockout animal models, means that defining potential therapeutic uses for PTP inhibitors has been difficult. The most convincing evidence for a useful therapeutic role of PTP inhibitors was the 1999 discovery that a PTP1B knockout mouse resulted in enhanced insulin sensitivity coupled with obesity resistance to a high fat diet, correlating with increased insulin-stimulated phosphorylation of the insulin receptor tyrosine [89]. Merck and McGill University patented this transgenic mouse homozygous for a disrupted PTP1B gene [303]. The mouse findings were confirmed by another group, who observed that PTP1B-deficient mice had tissue-specific increased insulin sensitivity, with resistance to diet-induced obesity. Increased energy expenditure and a marked decrease in fat cell mass (but not fat cell number) were observed [90]. The results of the two animal studies have been summarised in a table [79]. An earlier report had found (via overexpression of PTP1B or C215S PTP1B in clonal cell lines) that PTP1B is a negative regulator of insulin- and insulin-like growth factor-1-stimulated signalling [91]. There is also evidence that PTP1B is involved in dephosphorylation of the EGFR but

even with hyperphosphorylated EGFR observed in PTP1B$^{-/-}$ fibroblasts, there is little overactivation of the downstream pathways [22]. Increased EGFR activity can lead to tumours.

Leukocyte antigen related (LAR) PTP has also been implicated in the insulin signalling pathway; overexpression of LAR in the muscles of transgenic mice causes insulin resistance [92]. Previous reports had indicated a role for LAR in dephosphorylation of the regulatory domain of insulin receptor kinase and suppression of insulin receptor activation [93-95]. Transgenic mice deficient in LAR PTP have shown varying effects, including impaired mammary gland development and function, despite otherwise normal growth and development [96], neuronal defects [97] and reduced body weight accompanied by reduced plasma levels of insulin and glucose [98], although there is some debate about the latter results [79]. LAR has been patented for use in the treatment of diseases such as cancer and metastases, as it was observed that increased expression of hPTP LAR decreased formation of tumours *in vivo* [304].

The above results suggest that insulin signalling can be enhanced by specific inhibition of PTP1B (and/or potentially LAR), providing a treatment for insulin-resistance diseases such as Type II diabetes mellitus, by maintaining insulin receptors in the active (tyrosine phosphorylated) form [79,88,99,100]. Fuel metabolism may also be affected, leading to treatments for obesity. These findings have resulted in the majority of the effort at PTP inhibition focusing on the PTP1B enzyme. Most patents claiming PTP1B inhibitors claim for their use in treating disorders related to insulin resistance, hyperglycaemia or glucose intolerance, Type II diabetes, modulating glucose levels and obesity (and related disorders, such as hypertension, hypercholesterolaemia or hypertriglyceridaemia). Some patents also claim the treatment of atherosclerosis, cancer, neurodegenerative diseases (such as Alzheimer's disease) and growth hormone diseases.

The roles of other PTPs are less obvious, with biological studies demonstrating multiple possibilities. CD45 is an RPTP expressed on all haematopoietic cells, except erythrocytes, and is required for signal transduction leading to activation of B and T cells via their antigen-specific receptors [16]. CD45 targets the Src family of PTKs, and dephosphorylates two tyrosyl residues involved in PTK regulation [101]. CD45-deficient humans have a severe combined immunodeficiency (SCID) syndrome [102,103], while CD45-deficient mice show abnormal B and T cell development and function [104,105]. CD45 inhibitors are patented for treatment of immunological-related diseases, such as organ rejection, autoimmune disorders and anti-inflammatory agents. The role of CD45 in regulation of immune cell function was reviewed in 2000 [101].

SHP-1 regulates multiple signalling pathways in haematopoietic cells. A murine model of SHP-1 deficiency, the naturally-occurring *motheaten* (*me/me*) mouse, shows many haematopoietic abnormalities that result in death within 2 – 3 weeks [106]. A decrease in SHP-1 activity has been patented for the treatment of infection, inflammation and tumours. The structurally similar SHP-2 (55% overall sequence identity)

seems to possess alternate functionality to SHP-1 [2] and has been shown to be involved in the leptin signalling pathway [51], in regulating cell spreading, migration and focal adhesion [107] and in regulating early events in integrin signalling [108]. SHP-2 was recently reported to be an intracellular target of *Helicobacter pylori* Cag A protein, which is associated with severe gastritis and gastric carcinoma. The protein is injected from *H. pylori* into host cells, where it is phosphorylated, then forms a CagA–SHP-2 complex that results in stimulation of phosphatase activity [109]. Most immune inhibitory receptors interact with either or both SHPs [53,110-112].

Activated PTKs have been implicated in cancer and it has been suggested that PTP genes could act as tumour suppressor genes via production of PTPs that dephosphorylate over-phosphorylated proteins [19]. The phosphatases that dephosphorylate MAPK members have been comprehensively reviewed [17,113-115]. Both DSPs and classical PTPs can deactivate MAPKs. VHR appears to be a general MAPK phosphatase with a preference for hydrolysis of a pTyr residue [76]. Cdc25 protein phosphatases have been implicated in cell proliferation, as reviewed in 1997 [116], with both cdc25A and cdc25B overexpressed in cell tumour lines. Patents describing cdc25 inhibitors claim indications for cancer treatment. Phosphatase and tensin homologue deleted on chromosome ten (PTEN), a putative PTP gene, is mutated in human brain, breast and prostate cancer [117,118]. The PTEN phosphatase is a DSP. However, it actually dephosphorylates the sugar ring of phosphatidylinositol, rather than pTyr/pThr in protein substrates [22]. A tumour suppressor gene coding for a 47 kDa protein containing a PTP domain has been patented [305]. A patent by McGill University claims that the absence of TCPTP causes a delay in cell cycle progression at the G_0 stage and that the absence of TCPTP confers extreme sensitivity to DNA damaging reagents. The patent also claims that increased levels of TCPTP are detected in 70% of 14 colon cancer cases, meaning TC-PTP inhibitors could be useful for anticancer treatment [306]. TCPTP$^{-/-}$ mice die within 3 - 5 weeks, due largely to abnormal marrow stromal cells [124]. TCPTP and its gene were reviewed in 2000 [125].

Other PTPs have been knocked out in mice; lack of PTP-δ (expressed in specialised regions of the brain) causes impaired learning and enhanced hippocampal long-term potentiation and was semi-lethal due to insufficient food intake [119]. PTP-σ deficiency results in neuronal defects, causing early death or stunted growth with neurological defects [120,121]. PTP-ε (strongly expressed in the nervous system) deficient mice showed hypomyelination and hyper-phosphorylation of voltage-gated potassium (Kv) channel α-subunits [39]. PTP-ε is expressed as both a cytosolic isform (PTP-εC) and a transmembrane form (PTP-εM). PTP-εC has recently been identified as selectively inhibiting the IL-6 and IL-10-induced JAK-STAT signalling pathway [122]. PTP-ε has been implicated in bone resorption: alendronate is used clinically for treatment of osteoporosis and has been found to inhibit this PTP [123].

Yersinia PTP was one of the first PTPs identified and it is of interest as the phosphatase is essential for the pathogenesis of the organism responsible for the Black Death plague [126]. A number of patents related to PTPs themselves have been published in the past three years. Sugen has patented a number of novel phosphatases identified from the Celera human genomic sequence database, including PTPs [307], while Human Genome Science has published a similar patent claiming only PTPs [308]. Other patents have been filed for a polynucleotide encoding a PTP-like enzyme polypeptide [309], an isolated nucleic acid encoding rPTP 11 polypeptide [310], a dual specificity PTP (DSP-12) [311], two new hPTPs from human cancer cells [312] and an isolated rPTP [313]. Cardiovascular system associated PTPs (CSAPTP) have been patented by Millenium, who claim that treatment could be useful for immune disorder, cancer, diabetes, neural disease and diabetes; expression of CSAPT-1 affects the transcription level of a number of other genes [314].
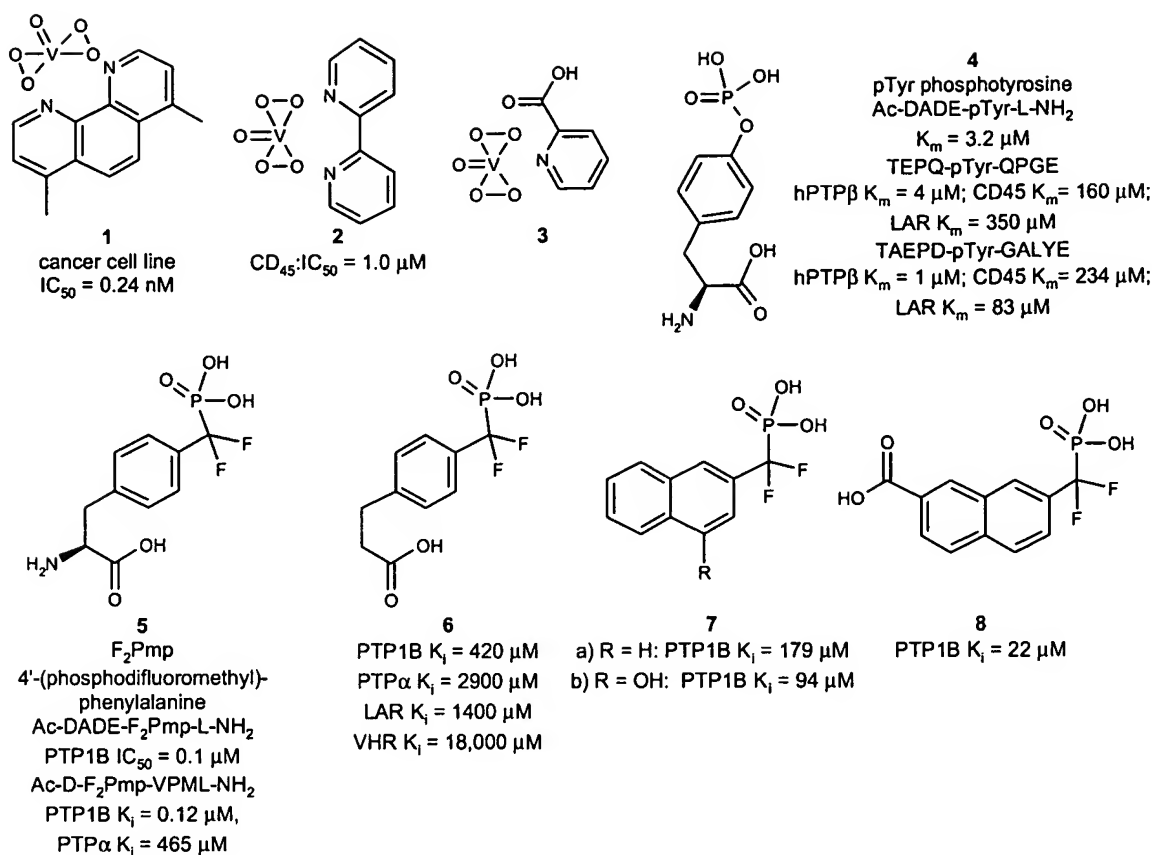
2. Discussion

2.1 Development of PTP inhibitors

The development of PTP inhibitors is at a nascent stage compared to PTK inhibitors [127]. PTK inhibitors, such as trastuzumab (Herceptin™, Genentech Corp.; a monoclonal antibody targeting the HER-2 transmembrane tyrosine kinase receptor for treatment of breast and other cancers) and imatinib (Gleevec™, Novartis AG; a small molecule selective inhibitor of Brc-Abl, c-Kit and PDGFr tyrosine kinases for treatment of chronic myeloid leukaemia) are already in clinical use. In contrast, there are very few PTP inhibitors that have even advanced into clinical trials. Several reviews of PTP inhibitors have been published [55,61,79,88].

2.2 Early PTP inhibitors

The first phosphatase inhibitors were non-competitive general inhibitors of low activity and minimal specificity, such as the metals molybdate (PTP1B, [20]), zinc (IC_{50} for a membrane PTP < 10 μM [128]; PTP1B IC_{50} = 15 μM [20]) and phenylarsine oxide (PTP-ε IC_{50} = 18 μM [123]; CD45 IC_{50} = 7 μM [129]). The acidic polymers heparin (PTP1B IC_{50} = 20 nM [20]) or 1:1 or 4:1 poly (Glu–Tyr) (PTP1B IC_{50} = 50 nM [20]) have high activity, but are also non-competitive inhibitors [20]. Sodium tungstate is a competitive inhibitor with K_d = 61 μM at pH 7 for *Yersinia* PTP [68,69,126] and IC_{50} = 10 μM for PTP1B [57]; crystal structures for both of these complexes were reported. Sodium phosphate itself inhibits TCPTP with IC_{50} = 15 mM; sodium thiophosphate has much stronger inhibition of IC_{50} = 470 μM [130]. Gallium nitrate inhibits a cellular membrane PTPase from Jurkat human T cell leukaemia cells (IC_{50} 2 - 6 μM), but not CD45 or cdc25 [131]. Vanadate has been found to be an effective inhibitor of PTPs with a variety of inhibition constants reported, for example, CD45 IC_{50} = 34.2 μM [123] or 80 μM [199], LAR IC_{50} = 30.8 μM [123], PTP1B IC_{50} = 30.2 μM [123], PTP-S2 IC_{50} = 38.1 μM [123], PTPε IC_{50} =

1
cancer cell line
IC_{50} = 0.24 nM

2
CD_{45}:IC_{50} = 1.0 µM

3

4
pTyr phosphotyrosine
Ac-DADE-pTyr-L-NH$_2$
K_m = 3.2 µM
TEPQ-pTyr-QPGE
hPTPβ K_m = 4 µM; CD45 K_m = 160 µM;
LAR K_m = 350 µM
TAEPD-pTyr-GALYE
hPTPβ K_m = 1 µM; CD45 K_m = 234 µM;
LAR K_m = 83 µM

5
F$_2$Pmp
4'-(phosphodifluoromethyl)-
phenylalanine
Ac-DADE-F$_2$Pmp-L-NH$_2$
PTP1B IC_{50} = 0.1 µM
Ac-D-F$_2$Pmp-VPML-NH$_2$
PTP1B K_i = 0.12 µM,
PTPα K_i = 465 µM

6
PTP1B K_i = 420 µM
PTPα K_i = 2900 µM
LAR K_i = 1400 µM
VHR K_i = 18,000 µM

7
a) R = H: PTP1B K_i = 179 µM
b) R = OH: PTP1B K_i = 94 µM

8
PTP1B K_i = 22 µM

0.3 µM [123] and VHR IC_{50} = 105.8 µM [132]. A membrane PTP from A-431 cells [133] and a PTP from TCRC-2 cells [134] have also been inhibited. Vanadate binds to the catalytic site of PTP1B and inhibits catalysis, with K_i = 81 µM for inhibition of binding of PTP1B with human IR by a scintillation proximity assay and K_i = 70 µM for inhibition of catalysis of hydrolysis of p-nitrophenol phosphate [85].

The mechanisms of PTP inhibition by vanadate and pervanadate were investigated by a comprehensive study in 1997 [135]. Vanadate was determined to be a competitive inhibitor of PTP1B (for hydrolysis of fluorescein diphosphate) with K_i = 0.38 µM, with inhibition reversed by addition of the metal chelator EDTA. In contrast, pervanadate was determined to be an irreversible inhibitor that oxidises the active site cysteine residue. Inhibition by either species was found to be very sensitive to the assay conditions and the vanadate–pervanadate can interconvert, further complicating the analysis of assay results [135]. Peroxyvanadium compounds with complexing ligands have been patented for the treatment of cancer, with compound 1 showing IC_{50} = 0.24 nM against a drug-resistant NIH ADR breast cancer cell line and significantly inhibiting tumour growth in an *in vivo* rat model [315]. The ligands influence phosphatase specificity, while the peroxyvanadate shows greater specificity against PTPs than vanadium salts. Similar bis-peroxyvanadium compounds, such as 2 and 3, have also been claimed for the treatment of immune disorders caused by viruses, via activation of T cells [316] and

as inhibitors of angiogenesis, restenosis and endothelin production [317]. Vanadyl sulfate was patented in combination with α-lipoic acid and taurine as a dietary supplement for the treatment of diabetes and obesity [380].

2.3 Phosphotyrosine mimetics and peptidic PTP inhibitors

Much effort in developing inhibitors of PTKs, PTPs and SH2 domains has focused on the synthesis of phosphotyrosine mimetics, which act as non-hydrolysable replacements for the critical pTyr (4) residue. Similar structures have been applied to all three programmes. Phosphotyrosyl mimetics were reviewed in a 2001 paper [136]. Over 30 phosphorus-containing pTyr mimetics, di-ionic non-phosphorus-containing pTyr mimetics, mono-ionic non-phosphorus-containing pTyr mimetics or non-ionic non-phosphorus-containing pTyr mimetics are discussed, although only some of these have been applied to PTP inhibitors. The article also addresses design considerations in obtaining drug-like PTP-directed pTyr mimetics; replacement of the ionised phosphonate species is critical in order to improve the cellular penetration of potential drugs.

Attempts to mimic pTyr residues have led to several classes of small molecule PTP inhibitors. One of the most effective pTyr analogues is the non-hydrolysable analogue 4'-phosphonyldifluoromethyl-phenylalanine, F$_2$PmP (5), which gives potent PTP inhibitors when incorporated into peptides [137,147]. Removing the α-amino group of F$_2$PmP
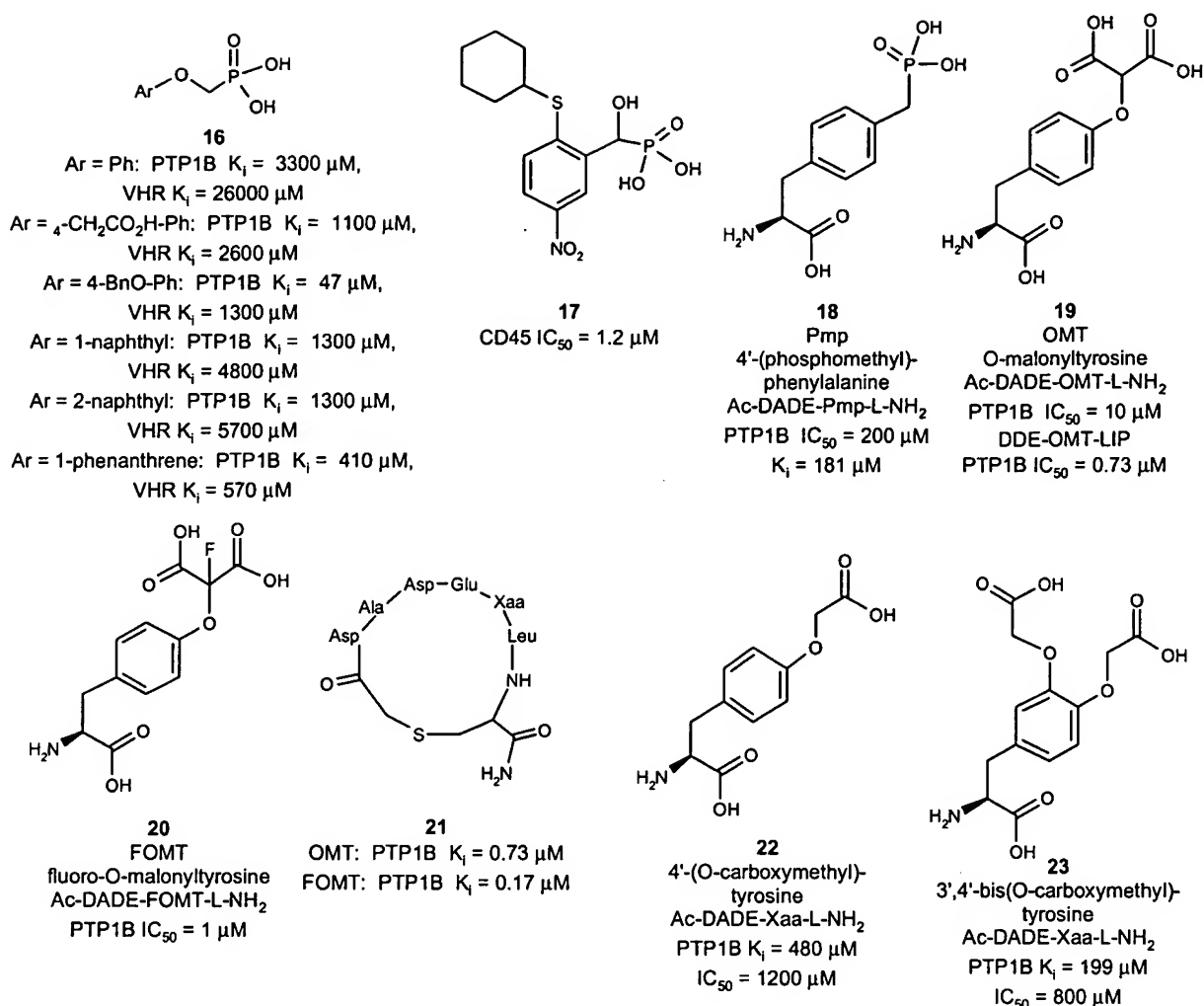
9
PTP1B K_i = 12 μM

10
3'-carboxy-4'-(O-carboxymethyl)-tyrosine
Ac-DADE-Xaa-L-NH₂
PTP1B K_i = 3.6 μM
IC_{50} = 19 μM

11 PTP1B IC_{50} = 250 μM

12
PTP1B IC_{50} = 900 μM

13
PTP1B IC_{50} = 31 μM

14
X = CF₂PO₃H: PTP1B IC_{50} = 35 μM
X = CF₂SO₃H: IC_{50} = 175 μM
X = CF₂CO₂H: IC_{50} = 640 μM
X = CF₂(5-tetrazolyl): IC_{50} = 230 μM
X = OCF(CO₂H)₂: IC_{50} = 320 μM

15
X = CF₂PO₃H: PTP1B IC_{50} = 15 μM
X = CF₂SO₃H: IC_{50} = 115 μM
X = CF₂CO₂H: IC_{50} = 435 μM
X = CF₂(5-tetrazolyl): IC_{50} = 195 μM
X = OCF(CO₂H)₂: IC_{50} = 250 μM

gives compound 6, with PTP1B K_i = 420 μM, selective against VHR, LAT and PTPα (K_i = 18,000, 1400 and 29000 μM, respectively) [138]. Another non-amino acid analogue of F₂Pmp adds the difluorophosphonomethyl moiety onto a napthalene ring (7a) (PTP1B K_i = 179 μM) [139]. A crystal structure has been reported [59]. Adding a hydroxy group to the napthyl ring to give compound 7b doubled the affinity (PTP1B K_i = 94 μM) [59,140], while adding an acid group designed to interact with Arg47 of PTP1B gave 6-(phosphonodifluoromethyl)-2-naphthoic acid (8) with PTP1B K_i = 22 μM [141]. This was converted into compound 9, a pseudo-dipeptide mimetic of Ac-E-pTyr-NH₂, by coupling the acid with Glu-NH₂, improving activity to PTP1B K_i = 12.4 μM [141]; a crystal structure with PTP1B was obtained [60].

3-Carboxy-4-(O-carboxymethyl)-L-Tyr (10) is another effective pTyr mimetic. As for F₂Pmp, this phosphonate ester mimetic moiety was transferred to a naphthyl-based template, with the direct analogues (11) and (12) having PTP1B IC_{50} = 250 and 900 μM, respectively [142]. Attempts to form dipeptide analogues with these compounds resulted in greatly reduced activity, indicating that the naphthyl 2-carboxy group is binding in the catalytic site rather than the expected O-carboxymethyl group. Indeed, 6-carboxy-1-naphthoic acid (13) has PTP1B IC_{50} = 31 μM [142].

Another group studied benzylic α,α-difluorosulfonates, α,α-difluorotetrazole and α,α-difluorocarboxylates 14 and 15, and their non-fluorinated analogues, as potential

monoanionic phosphate bioisosteres by attaching them to a 2-naphthyl or *m*-biphenyl aryl group. They were compared with an O-fluoromalonyl phenol substituent. The fluorinated derivatives were much more active than the non-fluoroinated, but the α,α-difluorophosphonates were nearly an order of magnitude more active than the other isosteres [143]. Several years later a series of aryloxymethylphosphonates ArOCH₂PO₃H₂ (16) were evaluated for PTP1B inhibition; a 4-BnO-Ph aryl group gave the best results (K_i = 47 μM) [144]. A molecular modelling study examined these three types of PTP1B inhibitors, compounds 14, 15 and 16. The aryloxymethylphosphonates seem to be anchored by specific hydrogen bonds, with potency affected by hydrophobic interactions, cationic π-stacking and van der Waals contacts. For the benzylic α,α-difluorobenzyl phosphate isostere series, the biphenyl compounds show greater potency than the naphthyl derivatives, due to a cationic π-interaction with Lys116. For isosteres of the phosphonate group, polar surface area appears to give a good correlation with potency, with sulfonate and tetrazole groups providing the best interactions [145].

Another early attempt at mimicking a pTyr residue led to a series of α-hydroxybenzyl phosphonates, of which the best, 17, had IC_{50} = 1.2 μM for CD45 [146]. A variety of other small molecule inhibitors that incorporate similar aryl and acid functionalities to those described above, but which do not seem to have evolved explicitly from pTyr mimetics, are discussed in the following sections.

16

Ar = Ph: PTP1B K_i = 3300 μM,
VHR K_i = 26000 μM
Ar = $_4$-CH$_2$CO$_2$H-Ph: PTP1B K_i = 1100 μM,
VHR K_i = 2600 μM
Ar = 4-BnO-Ph: PTP1B K_i = 47 μM,
VHR K_i = 1300 μM
Ar = 1-naphthyl: PTP1B K_i = 1300 μM,
VHR K_i = 4800 μM
Ar = 2-naphthyl: PTP1B K_i = 1300 μM,
VHR K_i = 5700 μM
Ar = 1-phenanthrene: PTP1B K_i = 410 μM,
VHR K_i = 570 μM

17
CD45 IC$_{50}$ = 1.2 μM

18
Pmp
4'-(phosphomethyl)-
phenylalanine
Ac-DADE-Pmp-L-NH$_2$
PTP1B IC$_{50}$ = 200 μM
K_i = 181 μM

19
OMT
O-malonyltyrosine
Ac-DADE-OMT-L-NH$_2$
PTP1B IC$_{50}$ = 10 μM
DDE-OMT-LIP
PTP1B IC$_{50}$ = 0.73 μM

20
FOMT
fluoro-O-malonyltyrosine
Ac-DADE-FOMT-L-NH$_2$
PTP1B IC$_{50}$ = 1 μM

21
OMT: PTP1B K_i = 0.73 μM
FOMT: PTP1B K_i = 0.17 μM

22
4'-(O-carboxymethyl)-
tyrosine
Ac-DADE-Xaa-L-NH$_2$
PTP1B K_i = 480 μM
IC$_{50}$ = 1200 μM

23
3',4'-bis(O-carboxymethyl)-
tyrosine
Ac-DADE-Xaa-L-NH$_2$
PTP1B K_i = 199 μM
IC$_{50}$ = 800 μM

The pTyr mimetics generally have low activity on their own, which is significantly enhanced by inclusion in peptides. An Ac-DADE-Xaa-L-NH$_2$ hexapeptide containing Pmp (18) had an IC$_{50}$ = 200 μM for PTP1B-mediated dephosphorylation of phosphorylated insulin receptor. By replacing the PmP residue with F$_2$Pmp (5) a three orders of magnitude improvement in activity was obtained, with IC$_{50}$ = 100 nM [137]. A study examined the reasons for the superiority of F$_2$Pmp over the non-fluorinated Pmp as a pTyr analogue by looking at the interactions of several Pmp and F$_2$Pmp peptides with PTP1B, LAR and PTPα. F$_2$Pmp has an estimated second pKa value (5.1) much closer to pTyr (5.7) than Pmp (5.1), but binding studies of the peptides as a function of pH did not show any pH dependence [147]. Instead, the authors hypothesised that the fluorine atoms interact specifically with amino acid side chains in the PTP1B active site. The study found a hexapeptide Ac-D-F$_2$Pmp-VPML-NH$_2$ with PTP1B K_i = 0.12 μM, selective over PTPα (K_i = 465 μM) [147].

The Ac-DADE-Xaa-L-NH$_2$ hexapeptide with another pTyr mimetic, O-malonyltyrosine (OMT, 19), designed for potential ease of derivatisation as a diester prodrug to improve cell permeability, had an intermediate IC$_{50}$ value of 10 μM [148],

while monofluoromalonyltyrosine (FOMT) (20) in the peptide gave IC$_{50}$ = 1 μM [149,150]. Three types of cyclic peptides containing the DADE-OMT-L sequence were prepared in an attempt to improve resistance to proteolysis and restrict conformational freedom. The best derivative improved activity from IC$_{50}$ = 10 μM for the linear sequence to K_i = 0.73 μM for a c[-CH$_2$CO-D-A-D-E-OMT-Leu-Cys-NH$_2$] peptide (21), cyclised from an N-terminal acyl substituent to a C-terminal cysteine side chain [149]. The same cyclic peptide with an FOMT residue had PTP1B K_i = 0.17 μM [151]; a crystal structure with PTP1B was obtained [60].

4-(O-carboxymethyl)-L-Tyr (22), 3,4-bis(O-carboxymethyl)-L-Tyr (23) and 3-carboxy-4-(O-carboxymethyl)-L-Tyr (10) were also incorporated into Ac-DADE-Xaa-L-NH$_2$ hexapeptides; the 3-carboxy-4-(O-carboxymethyl)-L-Tyr derivative had the best activity with K_i = 3.6 μM against PTP1B, comparable to the K_m of the corresponding pTyr-containing peptide [152]. In another study, 14 different mono- and di-carboxylic acid pTyr analogues (24) and (25) were incorporated into the Ac-DADE-Xaa-L-NH$_2$ hexapeptide; the best results were given by FOMT (20) with PTP1B IC$_{50}$ = 1 μM [153].
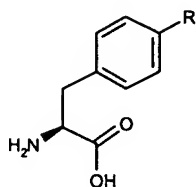
24
Ac-DADE-Xaa-L-NH$_2$
R =CO$_2$H: 4'-carboxy-Phe
PTP1B IC$_{50}$ = 4400 µM
R = CH$_2$CO$_2$H: 4'-carboxymethyl-Phe
PTP1B IC$_{50}$ = 2500 µM
R = CF$_2$CO$_2$H: 4'-carboxydifluoromethyl-Phe
PTP1B IC$_{50}$ = 650 µM
R = CH(CO$_2$H)$_2$: 4'-malonyl-Phe
PTP1B IC$_{50}$ = 1500 µM
R = CF(CO$_2$H)$_2$: 4'-fluoromalonyl-Phe
PTP1B IC$_{50}$ = 430 µM
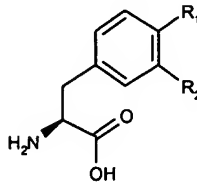
25
Ac-DADE-Xaa-L-NH$_2$
R$_1$ =CO$_2$H; R^2 = OH: 3'-hydroxy-4'-carboxy-Phe
PTP1B IC$_{50}$ = 5300 µM
R$_1$ = OH; R^2 = CO$_2$H: 3'-carboxy-4'-hydroxy-Phe
PTP1B IC$_{50}$ =4600 µM
R$_1$ = CH$_2$CO$_2$H; R^2 = OH: 3'-hydroxy-4'-carboxymethyl-Phe
PTP1B IC$_{50}$ = 13000 µM
R$_1$ = CH$_2$CO$_2$H; R^2 = CO$_2$H: 3'-carboxy-4'-carboxymethyl-Phe
PTP1B IC$_{50}$ = 3700 µM
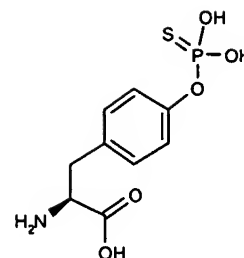
26
pTyr(S)
thiophosphotyrosine
TEPQpY(S)QPGE
hPTPβ KI = 96 µM; CD45 = K$_i$= 270 µM;
LAR K$_i$ = 1020 µM
TAEPDpY(S)GALYE
hPTPβ K$_i$ = 3 µM; CD45 = K$_i$= 330 µM;
LAR KI = 220 µM
ENDpY(S)INASL
TCPTP IC$_{50}$ = 0.5 µM
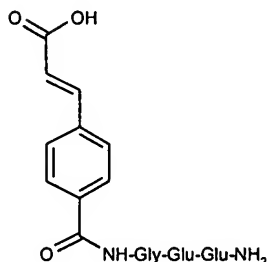
27
PTP1B IC$_{50}$ = 1.3 µM
K$_i$ = 0.079 µM
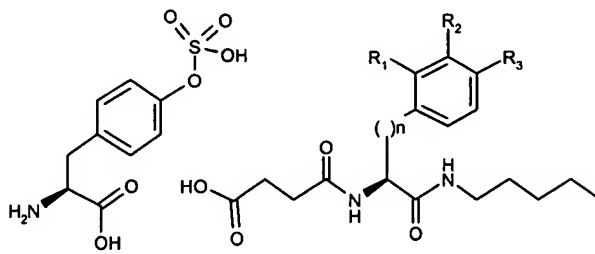
28
sY sulphotyrosine
TRDIsYEDTsYsYRK-NH$_2$
PTP1B: 90% inhibition at 5 µM
DIsYET
PTP1B IC$_{50}$ = 1 µM; CD45 IC$_{50}$ = 10 µM
EsYsY
PTP$_1$B IC$_{50}$ = 6 µM; CD45 IC$_{50}$ = 6 µM

29
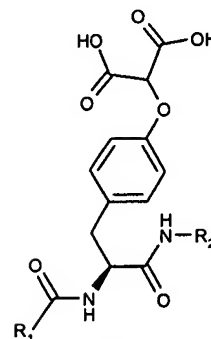
30
R$_1$ = CH(Bn)NHCO(CH$_2$)$_2$CO$_2$H;
R$_2$ = nPent
PTP1B K$_i$ = 0.7 µM
LAR: 26% inhibition at 100 µM
SHP-2: 71% inhibition at 100 µM

An important method for obtaining peptidic inhibitors is to map the subsite preferences of different PTPs using peptide substrates or inhibitors, providing information not only on which functional group moieties are required in different positions, but also on potential differences between enzymes to exploit for selectivity. The information from peptides can then be transferred to peptidomimetics or small molecules, especially if crystal structures of bound peptides are available. The substrate preferences of the catalytic domains of LAR, CD54 and hPTPβ have been reported [154], as have substrate specificities of Yersinia PTP and rat PTP1 [155]. In the first report three peptides containing thiophosphorylated tyrosine (26), a poorly hydrolysable pTyr analogue, were also prepared. They acted as inhibitors that were hydrolysed at a rate of 1 – 25% of that of the pTyr peptides and with K$_i$ values ~ 3 to 7-fold higher than the pTyr peptide K$_m$ values [154]. The

thiophosphorylated tyrosine (26) was also employed in an END-Xaa-INASL nonapeptide; TCPTP was inhibited with IC$_{50}$ = 0.5 µM [130]. Thiophosphorylated tyrosine has also been incorporated into a variety of peptide substrates of mammalian and bacterial transmembrane and intracellular PTPs, converting the substrates into inhibitors with IC$_{50}$ ≥ 0.23 µM (Yersinia PTP and PTP-1) and 0.75 µM (CD45) [198]. More recently, the subsite preferences of PTP1B were mapped by a 8000-member combinatorial chemistry library of octapeptides containing the pTyr analogue O-malonyltyrosine (mY) (19), in a DX$_3$X$_2$X$_1$(mY)LIP sequence [156]. Aromatic residues were preferred at the N-terminal proximal site X$_{-1}$, with acidic residues at X$_{-2}$ and X$_{-3}$. The most active peptide discovered by the Novartis group, DDEW(mY)LIP, had IC$_{50}$ = 0.73 ± 0.17 µM [156]. Ontogen used p-carboxycinnamic acid as the N-acyl group of a combinatorial tripeptide library; the most active
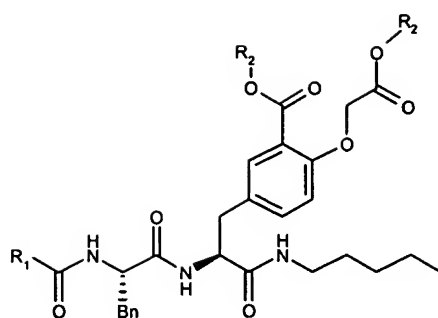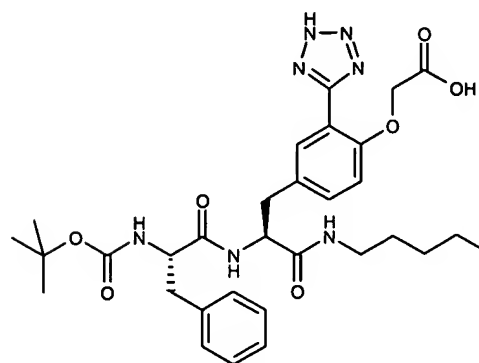
31
$R_1 = CH_2CH_2CO_2H$; $R_2 = H$
PTP1B $K_i = 0.22$ µM
LAR: 0% inhibition at 100 µM
SHP-2: 10% inhibition at 100 µM
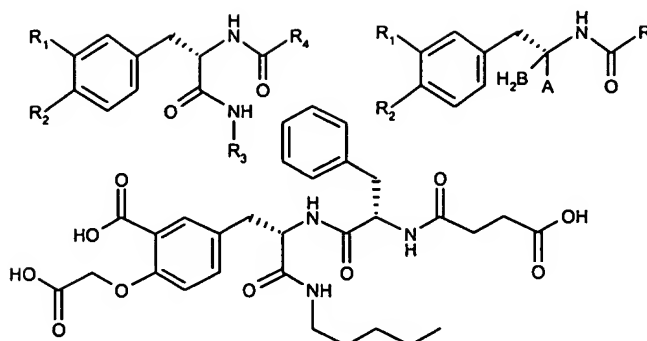
32
PTP1B: 64% inhibition at 1 µM
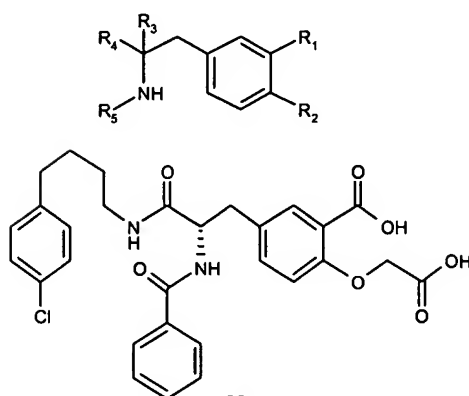
33
PTP1B $K_i = 0.87$ µM
97% inhibition at 100 µM

34
PTP1B: $K_i = 2.0$ µM
LAR: 0% at 100 µM
SHP-2: 0% at 100 µM

peptide (27) had a Gly-Glu-Glu-NH$_2$ sequence with PTP1B $IC_{50} = 1.3$ µM, $K_i = 0.079$ µM [157].

Sulfotyrosine (sTyr) (28) has been employed as a pTyr mimetic. A dodecapeptide corresponding to the 1142 – 1153 insulin receptor sequence was prepared with three of these residues and caused 90% inhibition of PTP1B at 5 µM; the sulfate ester was stable in the presence of PTP1B [158]. Another group obtained a DI(sY)ET pentapeptide with PTP1B $IC_{50} = 1$ µM (CD45 $IC_{50} = 10$ µM) and a E(sY)(sY) tripeptide with both PTP1B and CD45 $IC_{50} = 6$ µM [159]. Pharmacia identified a tripeptide lead compound Ac-Asp-Tyr(SO$_3$H)-Nle-NH$_2$ (PTP1B $K_i = 17$ µM) from screening octapeptide analogues of CCK-8 [64]. The tripeptide lead was first optimised by first evaluating a series N-succinyl pTyr mimetic pentylamides (29), then examining amidated/acylated derivatives of the best mimetics, O-malonyltyrosine (OMT) (30) and 3'-carboxy-4'-(O-carboxymethyl)tyrosine (31). The best compound inhibited PTP1B competitively with $K_i = 0.22$ µM and was selective versus LAR and SHP-2: data for TCPTP was not reported [64,65]. Crystal structures of PTP1B with both OMT-based and 3'-carboxy-4'-carboxy-Tyr-based compounds [64] and a truncated analogue [65] were

obtained. None of the most active compounds showed activity in a cell-based assay and they possessed very poor Caco-2 cell permeability values, as might be expected for multiply acidic compounds. Prodrug esters showed improved penetration and augmentation of insulin-stimulated 2-deoxyglucose uptake in cells [64,65]. Pharmacia & Upjohn patented these tyrosine and related derivatives as inhibitors of PTP1B, with acyl dipeptide amides such as compound 32 having 64% inhibition of PTP1B at 1 µM [318]. A similar patent containing structures such as compound 33 (PTP $K_i = 0.87$ µM) was published in 2000 [319]. A 2002 paper described replacement of the 3'-carboxy-4'-(O-carboxymethyl)tyrosine carboxyl groups with bioisosteres in order to improve cell permeability [66]. An ortho-tetrazole analogue (34) was equipotent to the derivative (31) with a dicarboxylic acid, both with PTP1B $K_i = 2$ µM (with selectivity over SHP and LAR but no selectivity over TC-PTP). The regioisomer, with the tetrazole replacing the acid group on the O-carboxymethyl moiety, was much less active. An X-ray cocrystal structure showed that the tetrazole moiety of compound 34 fits well in the active site. The compound had improved Caco-2 cell permeability compared to the dicarboxylic acid pTyr mimetics and showed

35
cdc25A $IC_{50} = 1.1\ \mu M$

36
BPPM
PTP1B $k_{cat} = 6.9\ s^{-1}$, $K_m = 16\ \mu M$

37

PTP1B $K_i = 0.93\ \mu M$
PTPα $K_i = 120\ \mu M$
LAR $K_i = 100\ \mu M$
VHR $K_i = 3600\ \mu M$

PTP1B $K_i = 1.4\ \mu M$
PTPα $K_i = 1300\ \mu M$
LAR $K_i = 60\ \mu M$
VHR $K_i = 3100\ \mu M$

38
PTP1B $IC_{50} = 4.4\ \mu M$
TCPTP $IC_{50} = 6.4\ \mu M$
SHP-1 $IC_{50} = 31\ \mu M$
SHP-2 $IC_{50} = 91\ \mu M$
CD45 $IC_{50} = 198\ \mu M$
Cdc25a $IC_{50} = > 250\ \mu M$
PTPβ $IC_{50} = 342\ \mu M$

39
PTP1B $IC_{50} = 26\ \mu M$

40
X = O(CH$_2$)$_2$O, O(o-Ph)O,
O(m-Ph)O, O(p-Ph)O
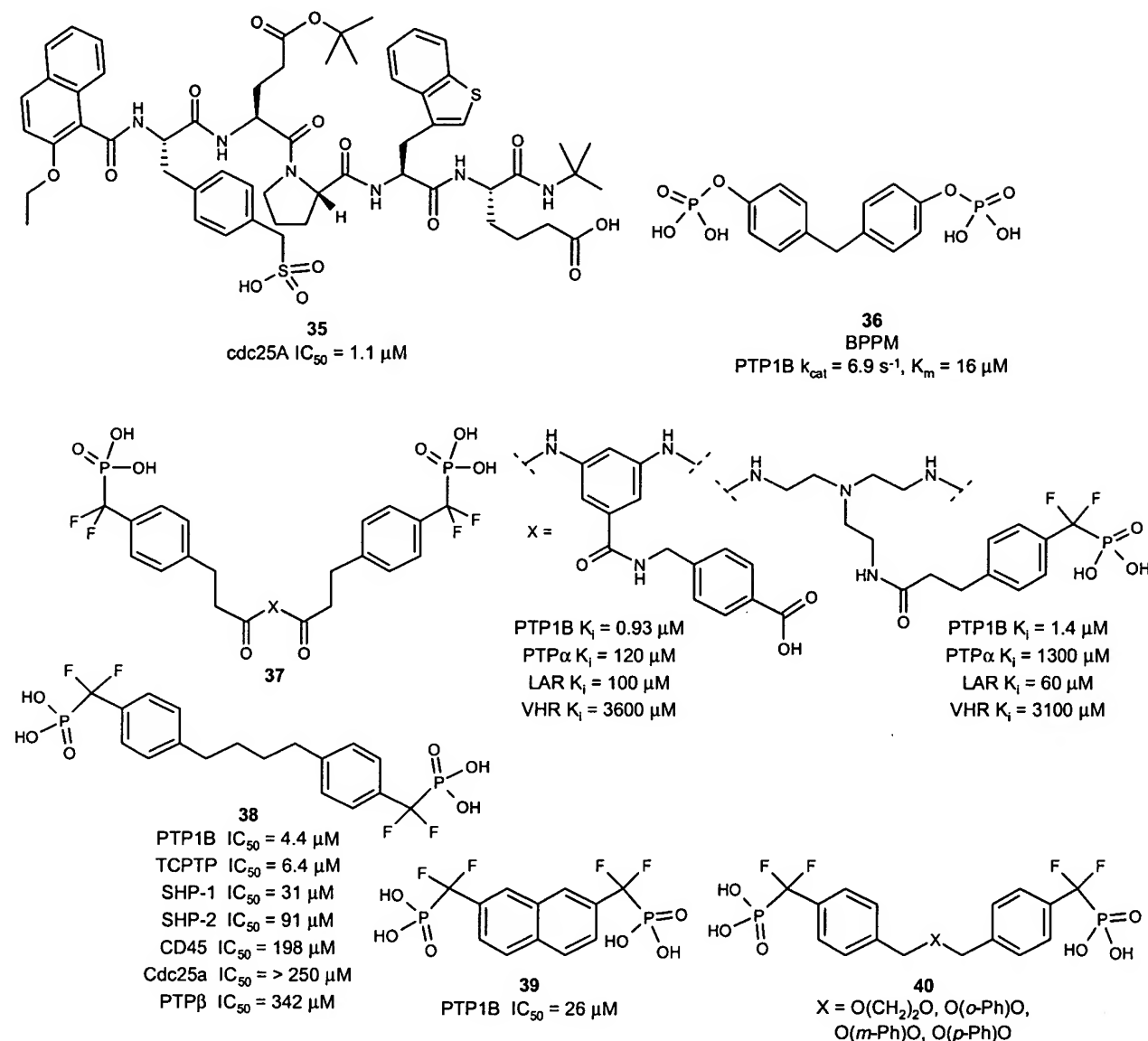
enhancement of insulin-stimulated 2-deoxyglucose uptake in L6 myocyte cells [66].

BASF published a patent describing peptidic inhibitors of cdc25A, with a pentapeptide (35) containing the sulfated pTyr mimetic 4'-sulphonomethyl-Phe possessing cdc25A $IC_{50} = 1.1$ μM [320]. The patent also claimed for crystalline cdc25B and cdc25C proteins and a method to determine their three-dimensional structure for use in identifying inhibitors.

The discovery that PTP1B has a binding site that accommodates a second pTyr residue adjacent to the catalytic site pTyr residue (see Section 1.2) [33,38] has led to the development of a number of bis(pTyr) analogue inhibitors targeted to PTP1B. Bis-(p-phosphophenyl)methane BPPM (36) is a PTP1B substrate with $k_{cat} = 6.9\ s^{-1}$ and $K_m = 16\ \mu M$, which has ~ 300-fold stronger affinity than pTyr) [33,160]. However, a single BPPM binds separately, but mutually exclusively, to each site rather than binding to both sites as a divalent single molecule [33]. A

series of bis(aryldiflorophosphonates) (37) were prepared to take advantage of the PTP1B structural feature, joining two deaminated F$_2$Pmp moieties via 20 different linkers. A meta-substituted diaminophenyl linker gave PTP1B $K_i = 0.93\ \mu M$ [138]. An tris(2-aminoethyl)amine-linked trimer also showed good potency (PTP $K_i = 1.4\ \mu M$)[138].

Merck Frosst (Canada) has devoted much of its PTP research effort towards bis(phosphonate) derivatives. A collaboration between the University of Waterloo, Merck Frosst (Canada) and Queens University [61] determined the crystal structure of PTP1B bound with two non-peptidyl aryl bis(difluoromethyl-enephosphonate) inhibitors, compounds 38 and 39, that had been proposed to simultaneously bind to both the active site and adjacent non-catalytic binding site, even though one of the compounds has too short a distance between the difluoromethylenephosphonate groups for them to simultaneously reach both sites. Neither compound bound as a bidentate ligand.
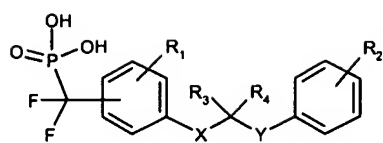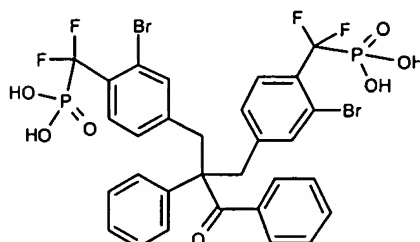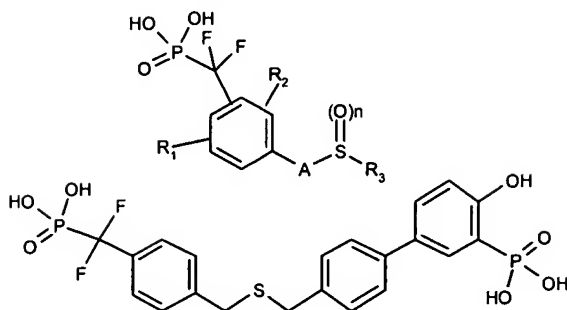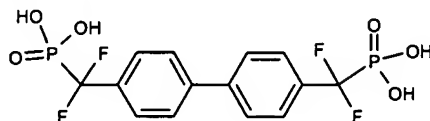
41
no biological data
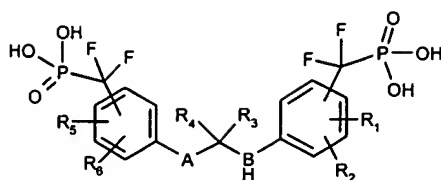
42
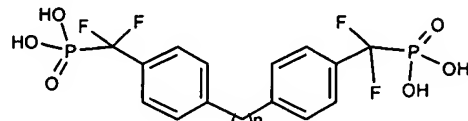no biological data
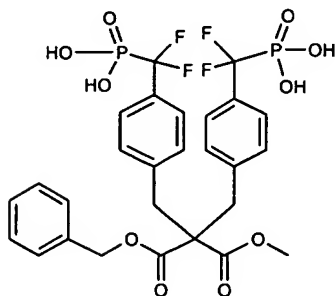
43
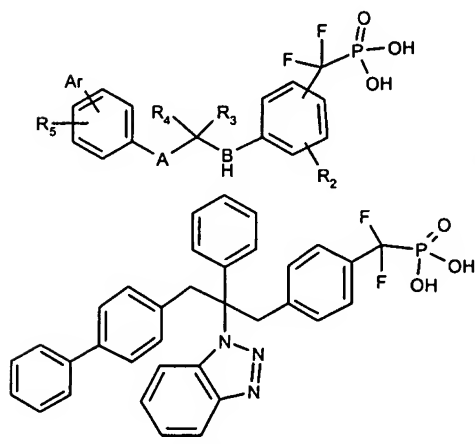no biological data

44
no biological data

45
R = *m*-Ph
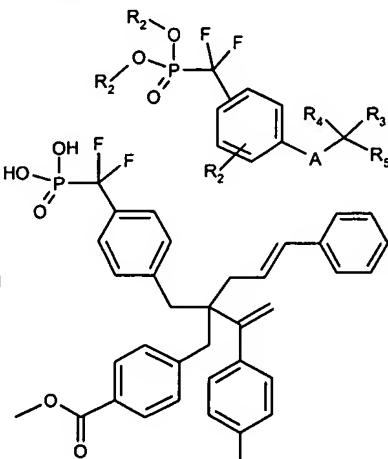PTP1B: 94% inhibition at 500 µM

46
PTP1B: IC_{50} = 9.9 µM
K_i = 4.5 µM

47
n = 4:
PTP1B: IC_{50} = 4.4 µM
K_i = 1.5 µM

48
no biological data

49
no biological data

50
no biological data

51
PTP1B; no activity reported

52
a) R = CF$_2$PO$_3$H$_2$
PTP1B IC$_{50}$ = 0. 004 μM
CD45 IC$_{50}$ = 33 μM
b) R = OH
PTP1B IC$_{50}$ = 0.004 μM
CD45 IC$_{50}$ = > 50 μM

53
a) R = L-Glu:
PTP1B IC$_{50}$ = 0.04 μM
LAR IC$_{50}$ = 45 μM
CD45 IC$_{50}$ = 7.5 μM
PTPβ IC$_{50}$ = 4.2 μM
SHP-1 IC$_{50}$ = 6 μM
b) R = L-Pro
PTP1B IC$_{50}$ = 0.3 μM
LAR IC$_{50}$ = 100 μM
CD45 IC$_{50}$ = > 100 μM
PTPβ IC$_{50}$ = 0.2 μM
SHP-1 IC$_{50}$ = > 10 μM

54
Alendronate
PTPε IC$_{50}$ = 2 μM

55
Dysidiolide
Cdc25A IC$_{50}$ = 9.4 μM
no inhibition of LAR, CD45 at 12.4 μM
IC$_{50}$ = 4.7 μM for A-549 human lung carcinoma
IC$_{50}$ = 1.5 μM for P-388 murine leukaemia

56
Cdc25A IC$_{50}$ = 2.2 μM

Other analogues with alternate linkers (40) were also examined; none showed selectivity for PTP1B over the closely related TCPTP [61]. Selectivity is highly desirable as TCPTP knockout mice die soon after birth [124]. A Merck patent claimed these linked aryl group diacids and related compounds for inhibition of PTP1B, including 2,3-bis(4-[difluoro(phosphono)methyl]-benzyl)malonic acid monobenzyl monomethyl ester (41), although no biological data were disclosed [321]. Other patents described bis(aryldifluoromethylphosphonic acids), such as compound 42 [322], or aryldifluoromethylphosphonic acids with a sulfur substituent, such as bis(aryldifluoromethylphosphonic acids) connected by a sulfur-based linker (43) [323] and similar naphthyldifluoromethylphosphonic acid-based compounds (44) [324].

A collaboration between Merck and the University of Toronto examined the inhibition of PTP1B by aryl-substituted aryl-α,α-difluoromethylenephosphonates (45), with a meta-phenyl substituent showing the best activity (94% inhibition at 500 μM) [161]. Dimerising these inhibitors generally increased potency, with compound 46 possessing PTP1B IC$_{50}$ = 9.9 μM [161]. Adding a butane linker between the two α,α-difluorobenzylphosphonate moieties gave an inhibitor (47) with PTP1B K$_i$ = 1.5 μM [161]. A series of Merck patents claimed PTP1B inhibitor compounds with an aryldifluoromethylphosphonic acid moiety linked to a biaryl group, such as compound 48 [325], and for other compounds with an aryldifluoromethylphosphonic acid moiety, such as compounds 49 [326] and 50 [327].

Merck has also claimed dipeptide inhibitors, including two adjacent acid-substituted Phe residues, such as compound 51, for the treatment of PTP1B-mediated diseases [328]. No examples of activity are presented in the patent, but at a conference presentation (S,S)-Bz-F$_2$Pmp-F$_2$Pmp-NH$_2$ (52) was reported to have PTP1B IC$_{50}$ = 4 nM activity (vs CD45 IC$_{50}$ = 33 μM).

57
Cdc25 IC$_{50}$ = 6 µg/mL
IC$_{50}$ lung adenocarcinoma (A549) cells = 7 µM
IC$_{50}$ colon cancer cells (HT-29) = 5 µg/mL

58
Dysidiolide analogue
Cdc25C IC$_{50}$ = 0.8 µM

59
Coscinosulfate
Cdc25B IC$_{50}$ = 3 µM

60
Sulfircin
PTP1B IC$_{50}$ = 29.8 µM
Cdc25A IC$_{50}$ = 7.8 µM
VHR IC$_{50}$ = 59 µM

61
PTP1B IC$_{50}$ = 21 µM
Cdc25A IC$_{50}$ = 6.3 µM
VHR IC$_{50}$ = 5.6 µM

62
2-methyl-fervenulone
95 - 100% inhibition of
PTPαD1D2, PTPεD1D2,
CD45, TCPTP, LAR-D1, at 1 µM

63
RK-682
CD45 IC$_{50}$ = 54 µM
VHR IC$_{50}$ = 2.0 µM

The other three diastereomers had PTP1B IC$_{50}$ = > 400 nM [162]. (*S,S*)-Bz-F$_2$Pmp-Tyr-NH$_2$ had similarly high activity (PTP1B IC$_{50}$ = 4 nM) and selectivity (CD45 IC$_{50}$ = > 50 µM). Selectivity against TCPTP was reported to be difficult to obtain [162]. Merck published a paper describing potent tripeptides with an Xaa-F$_2$Pmp-F$_2$Pmp-NH$_2$ motif. The Glu-F$_2$Pmp-F$_2$Pmp-NH$_2$ peptide (**53**) was selective for PTP1B over other PTPs (PTP1B IC$_{50}$ = 40 nM versus CD45 IC$_{50}$ = 7.5 µM, PTPβ IC$_{50}$ = 4.2 µM, LAR IC$_{50}$ = 45 µM, SHP-1 IC$_{50}$ = 6 µM). By changing the N-terminal residue to a proline, selectivity was changed in favour of PTPβ (PTP1B IC$_{50}$ = 0.3 µM, CD45 IC$_{50}$ = > 100 µM, PTPβ IC$_{50}$ = 0.2 µM, LAR IC$_{50}$ = 100 µM, SHP-1 IC$_{50}$ = > 10 µM). Other peptide sequence motifs (Xaa-F$_2$Pmp-Xaa-NH$_2$ and F$_2$Pmp-Xaa- F$_2$Pmp-NH$_2$) were less potent [163].

Alendronate (**54**) is an aminobisphosphonate that is used clinically to inhibit bone resorption. It has been found to inhibit PTPε with IC$_{50}$ = 2 µM [122].

## 2.4 PTP inhibitors from natural products and combinatorial libraries

A variety of small molecule PTP inhibitors have been identified from natural product screening programmes. Some of these act as mechanism-based inhibitors, so are discussed separately in Section 2.6. Marine sponges are a productive source for isolating novel natural products, including phosphatase inhibitors. Dysidiolide (**55**) is Cdc25A selective inhibitor (Cdc25A IC$_{50}$ = 9.4 µM; no inhibition of CD45 or LAR at 12 µM) with antitumour activity (IC$_{50}$ for inhibition of A-549 human lung carcinoma = 4.7 µM) that was isolated from a marine sponge [164]. It inspired a related compound (**56**) produced by pyrolysis of a cholesterol derivative, with Cdc25A IC$_{50}$ = 2.2 µM [165]. Related seco-cholestane derivatives such as compound **57** were patented by Georgia Tech. Res. as inhibitors of Cdc25 phosphatase activity with IC$_{50}$ = 0.9 to > 50 µg/ml; (**57**) had IC$_{50}$ = 6 µg/ml, showed a 50% reduction in growth of lung adenocarcinoma cells at 7 µM and possessed IC$_{50}$ = 5 µg/ml for proliferation of HT-29 colon cancer cells [329]. A number of dysidolide analogues were synthesised on a solid-phase support, with the most active compound (**58**) possessing Cdc25C IC$_{50}$ = 0.8 µM [166]. Coscinosulfate (**59**) is a sea sponge metabolite that also selectively inhibits Cdc25A (IC$_{50}$ = 3 µM) [167]. Sulfircin (**60**), isolated from a deep-water sponge, acts as a nonspecific PTP inhibitor [168]. A number of analogues were prepared with slightly improved or reduced activity; replacement of the unstable sulfate with a malonate gave an equipotent compound (**61**) [168].
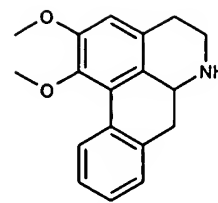
Streptomyces is another source of PTP inhibitors. 2-Methyl-fervenulone (**62**) is a non-selective PTP inhibitor isolated from a *Streptomyces* species extract that showed 95 - 100%
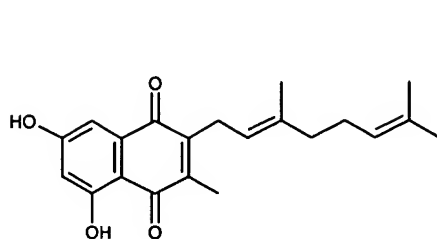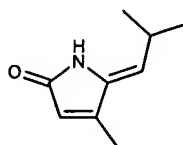
64
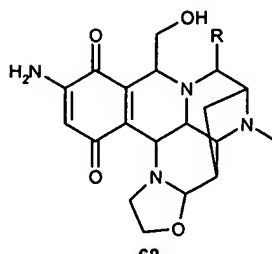Phosphatoquinone B
CD45 IC_{50} = 1 µg/ml, 2.9 µM
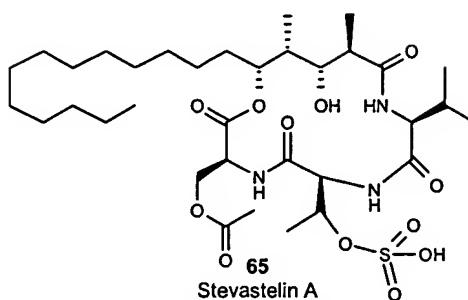
65
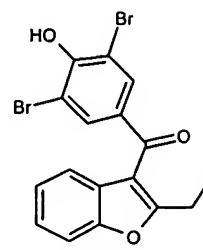Stevastelin A
VHR IC_{50} = 2.7 µM
CD45 IC_{50} = > 1000 µM

66
Nornuciferine
CD45 IC_{50} = 5.3 µM

67
Pulchellalactam
CD45 IC_{50} = 124 µg/ml, 800 µM

68
Dnacin
A1: R = CN cdc25B IC_{50} = 141 µM
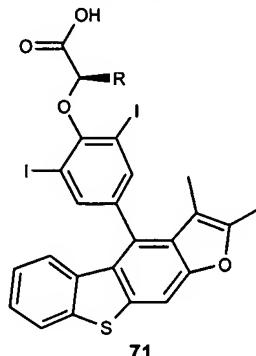B1: R = OH cdc25B IC_{50} = 64.4 µM

69
Benzbromarone
rat PTP1B IC_{50} = 26 µM
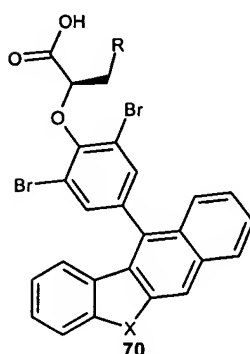
70
a) X = S; R = CH_2(phthalimide)
PTP1B IC_{50} = 0.011 µM
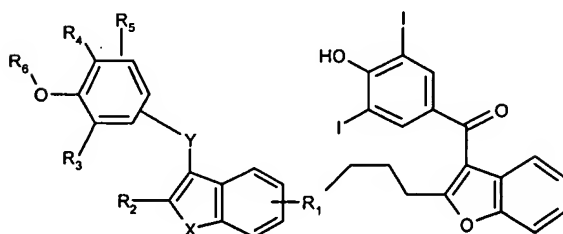no activity in ob/ob mice
b) X = O; R = Ph
PTP1B IC_{50} = 0.083 µM, LAR IC_{50} = 0.42 µM,
CD45 IC_{50} = 6.3 µM, PTP1a IC_{50} = 13 µM,
PTP-PEST IC_{50} = 2.2 µM, VHR IC_{50} = 11 µM
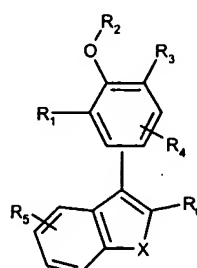42% decresed in glucose, 86% decrease in insulin
in ob/ob mice at 25 mg/kg/day p.o.

71
R = Me: PTP1B IC_{50} = 0.284 µM
R = Bn: PTP1B IC_{50} = 0.074 µM
43% decrease in insulin in ob/ob
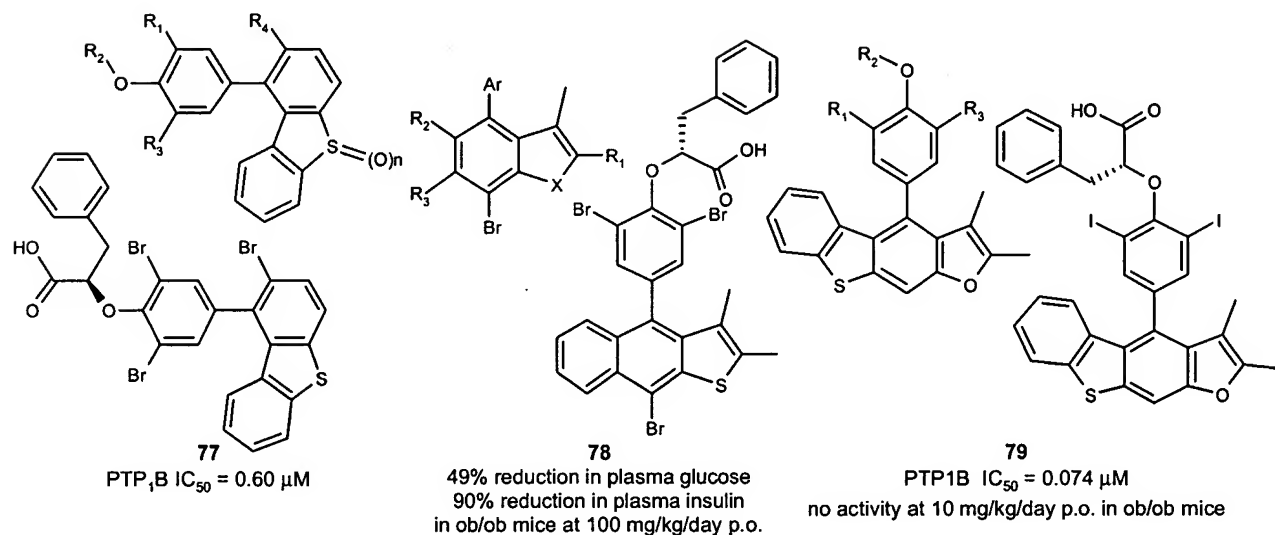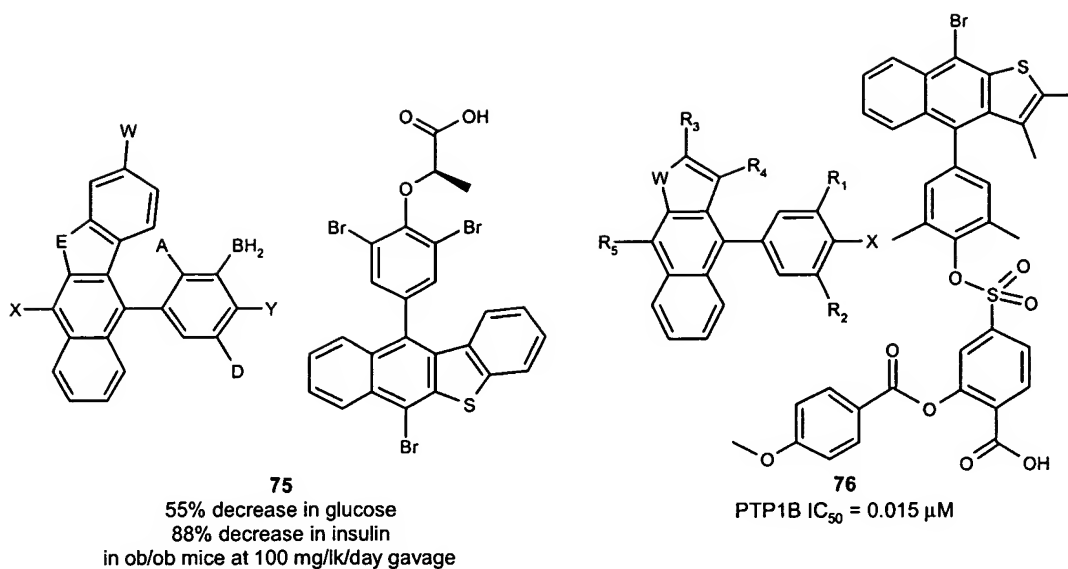mice at 10 mg/kg/day p.o.
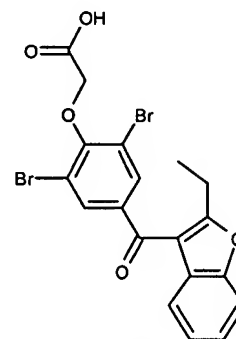
72
48% decrease in glucose in ob/ob
mice at 100 mg/lk/day gavage

73
52% decrease in glucose,
74% decrease in insulin
in ob/ob mice at100 mg/lk/day gavage

74
29% decrease in plasma glucose
13% decrease in plasma insulin
in ob/ob mice (100 mg/kg/day gavage dose)

75
55% decrease in glucose
88% decrease in insulin
in ob/ob mice at 100 mg/lk/day gavage

76
PTP1B IC_{50} = 0.015 µM

77
PTP_1B IC_{50} = 0.60 µM

78
49% reduction in plasma glucose
90% reduction in plasma insulin
in ob/ob mice at 100 mg/kg/day p.o.

79
PTP1B IC_{50} = 0.074 µM
no activity at 10 mg/kg/day p.o. in ob/ob mice

inhibition of CD45, LAR-D1, PTPαD1D2, PTPεD1D2 and TCPTP at 1 µM concentration [169]. RK-682, 3-hexade-canoyl-5-hydroxymethyltetronic acid (63), is a selective inhibitor of VHR (IC_{50} = 1-2 µM; CD45 IC_{50} = 54 µM; cdc25A, cdc25B IC_{50} > 100 µM) that was isolated from a *Streptomyces* species [170]. The compound arrested growth of Ball-1 cells at the G_1/S cell cycle stage [170,171]. A number of less potent analogues were synthesised; the long alkyl side chain is important for activity [171]. Rikagaku Kenykujo patented two bacterial metabolites, phosphatoquinones A and B, from a *Streptomyces* strain, with the more potent compound 64 inhibiting CD45 with IC_{50} of 1 µg/ml (2.9 µM) [330]. Their structural determination has been published [172].

Other natural product-derived inhibitors include stevaste-lin A (65), the sulfonylated derivative of stevastelin B (an immunosuppressant that inhibits gene expression; both isolated from the broth of *Penicillium* sp. NK374186). Stevaste-lin A is an inhibitor of the dual-specificity phosphatase VHR

(IC_{50} = 2.7 µM; > 1000 µM for CD45) [200]. Three CD45 inhibitors were isolated from extracts of the stem and stem-bark of *Rollinia ulei*, with nornuciferine (66) exhibiting the greatest potency (CD45 IC_{50} = 5.3 µM) [173]. Pulchellalactam (67) is a CD45 inhibitor (IC_{50} = 124 µg/ml, 800 µM) isolated from a marine fungus (*Corollospora pulchella*) found on a sample of driftwood from Peleliu [174]. Two antitumour antibiotics, dnacin A1 and B1 (68) are non-competitive inhibitors of cdc25B (IC_{50} = 141 and 64.4 µM, respectively [175].

A screening programme at Wyeth-Ayerst/American Home Products identified benzbromarone (69) as a rat PTP1B inhibitor (IC_{50} = 26 µM) with good oral absorption and pharmacokinetic properties. The lead was converted into potent compounds based on a 11-arylbenzo[b]naphtha[2,3-d]furan/thiophene template (70), giving compounds with *in vitro* potency as high as PTP1B K_i = 11 nM (70a). The most potent compound (70a) did not have activity in a diabetic ob/ob mouse model, but other analogues, such as compound
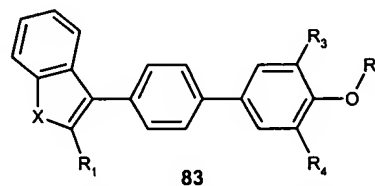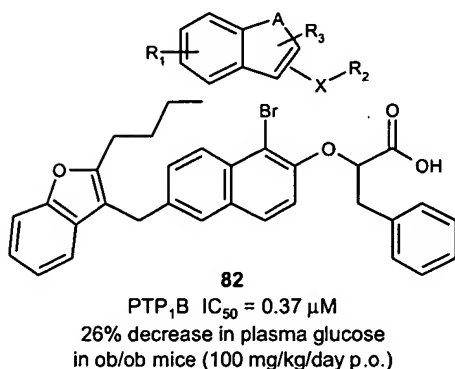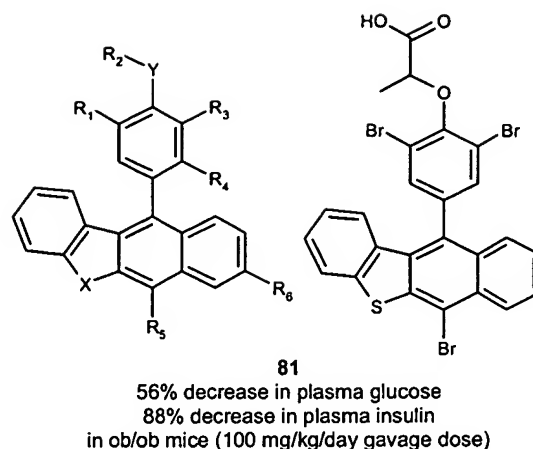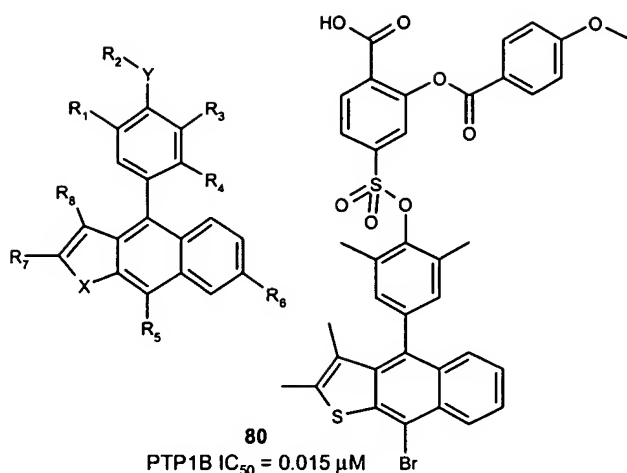
70b, did have such activity [176]. Further refinements included changing the naphthalene ring system to a benzofuran system to maximise a potential side chain interaction with Lys120, providing compounds such as 71 that retained nanomolar activity (PTP1B IC_{50} = 74 nM) but had improved *in vivo* activity in the diabetic ob/ob mouse, lowering insulin levels 43% at a 10 mg/kg/day p.o. dose [177]. These compounds and many others were patented by American Home Products in a series of very similar patents published in 1999 – 2001. The phenyloxoacetic acid and dihalophenol compounds that are related more closely to benzbromarone (69), such as compounds 72 and 73, are included in the first set of patents in 1999 [331]. Compounds 72 and 73 induced 48 and 52% reduction in glucose levels, respectively, in *in vivo* studies, with compound 73 also inducing a 74% reduction in insulin levels [331]. Similar compounds (74, 29% reduction on plasma glucose, 13% reduction in plasma insulin in ob/ob mice at 100 mg/kg/day) are subsequently claimed in a US patent [340]. Compounds with a benzo[b]naphtho[2,3-d]furan or benzo[b]naphtho[2,3-d]thiophene substituent off the phenol ring, such as compound 75 (54.6% decrease in glucose level, 88% decrease in insulin level in ob/ob mice at 100 mg/kg) are included in another patent [332], as are similar compounds without the extra fused benzene ring on the thiophene/furan, napth[2,3-b]heteroaryls, such as compound 76 (PTP1B IC_{50} = 0.015 µM) [333]. 1-Aryl-dibenzothiophenes without the extra benzene ring that forms the naphthyl group such as compound 77 are claimed in a US patent [334], while yet another patent is focused on benzothiophene, benzofuran and indole derivatives, such as compound 78 (49% reduction in blood glucose level in ob/ob mouse) [335,341].

Several patents claimed more specific structures, such as 4-aryl-1-oxa-9-thiacyclopenta[b]fluorene derivatives (79) (PTP1B IC_{50} = 0.074 µM, no activity in ob/ob mice at 10 mg/kg/day p.o. dose) [336], naptho[2,3-b]hetero-4-aryl derivatives (80) (PTP1B IC_{50} = 0.015 µM) [337] or benzonaphthyl derivatives with an extra fused benzene ring of compound 81 (55% reduction in plasma glucose and 88% reduction in plasma insulin in ob/ob mice at 100 mg/kg/day gavage dose) [338]. A general class of aryl-oxo-acetic acids (82) were claimed, focusing on bicyclic aryl moieties, such as naphthyl, benzothiaphene or benzofuryl for attachment of the oxoacetic acid substituent (PTP1B IC_{50} = 0.37 µM, 26% reduction in plasma glucose in ob/ob mice) [339].

The Wyeth-Ayerst group also identified benzofuran and benzothiophene-substituted biphenyl compounds (83). A phenol group on the biphenyl gave good activity. Replacing this with an oxoacetic acid group reduced activity 30fold, but a phenyllactic acid group gave an almost 3-fold improvement, which modelling suggests is due to interactions with the hydrophobic pocket near Arg221. The benzothiophenes tended to have better activity than the benzofurans. A crystal structure of one of the most potent compounds (83e) (PTP1B IC_{50} = 0.095 µM) with PTP1B indicated an additional potential pocket that could be used for a hydrophobic interaction with an *m*-aryl

substituent on the phenol. This led to the prediction that for bis(*m*-substituted) compounds an unsubstituted oxo acetic acid would be better than the oxo phenyllactic acid substituent, due to steric interactions. Indeed, compounds such as 83g with PTP1B IC_{50} = 25 nM activity were produced [63]. *In vivo*, the best compound was not the most potent PTP1B inhibitor; instead compound 83d (PTP1B IC_{50} = 0.32µM) reduced glucose levels in ob/ob mice by 27% at an oral dose of 25 mg/kg/day [63]. These compounds are included in the first set of Wyeth-Ayerst/American Home Products patents claiming biphenyloxoacetic acid derivatives, such as compound 84 (40% decrease in blood glucose levels and 45% decrease in insulin levels in ob/ob mice at 100 mg/kg/day gavage dosage) [342]. Another series of biphenyl compounds with potent activity (PTP IC_{50} = 24 nM) was obtained from a salicylic acid-sulfone on the phenol group (85) [63]; a crystal structure of one compound was also obtained. The biphenyl sulfonyl aryl carboxylic acids are included in another patent, with compound 86 reducing glucose levels by 25% in ob/ob mice at 25 mg/kg/day gavage dosing [343]. A patent describing furans, benzofurans and thiophenes attached via a linker to a phenol with an *O*-sulfone substituent (salicyclic acid), such as compound 87 (PTP1B IC_{50} = 0.060 µM) was claimed by American Home Products [344]. Biphenylsulfonyl aryl carboxylic acids with heteroaryl substituents off the biphenyl moiety (88) (PTP1B IC_{50} = 0.193 µM) are also described in a 2001 patent [345]. Another patent covering 2,3,5-trisubstituted biphenyl derivatives; with a long alkyl substituent (89), had PTP1B IC_{50} = 0.015 µM [346]. The same compounds were included in a United States patent [357]. A further patent describes oxazole arylcarboxylic acid derivatives, such as compound 90, with *in vivo* biological data in ob/ob mice showing a 40% decrease in blood glucose levels at 100 mg/kg/day gavage dosage for (90) [347]. The compounds are claimed to inhibit PTPs and the O-carboxymethyl phenol moiety certainly mimics a pTyr residue. American Home Products also claimed benzophenone based compounds, such as compound 91, with a benzoyl attachment to the phenolic pharmacophore [348]. The ester, presumably a prodrug, had lower activity compared to the acid.

Wyeth-Ayerst researchers have also identified a class of azolidinediones as possessing PTPase inhibition, in particular, those with an elongated spacer between the azolidinedione moiety and a central aromatic ring (92). Hydrophobic groups near the aromatic group were also important. Improvements in activity against PTP1B (e.g., PTP1B IC_{50} = 1, 9, 0.3 and 0.12 µM for a series of compounds) did not necessarily correlate with improved activity in an ob/ob mouse model (86, 30 and 0% reduction in insulin plasma levels, respectively), demonstrating the importance of ADME (Adsorption, distribution, metabolism, excretion) properties in obtaining useful biological activity [178]. A complicating factor in interpreting the *in vivo* results is that the authors caution that structurally similar thiazolidinediones have been identified as high affinity agonists of the peroxisome proliferator-activated receptor γ (PPARγ). The thiazolidinediones exhibit a direct correlation

80
PTP1B IC$_{50}$ = 0.015 µM

81
56% decrease in plasma glucose
88% decrease in plasma insulin
in ob/ob mice (100 mg/kg/day gavage dose)

82
PTP$_1$B IC$_{50}$ = 0.37 µM
26% decrease in plasma glucose
in ob/ob mice (100 mg/kg/day p.o.)

83
a) X = O; R1 = butyl; R$_2$ = R$_3$ = R$_4$ = H:
 PTP1B IC$_{50}$ = 0.74 µM
b) X = O; R$_1$ = butyl; R$_2$ = CH$_2$CO$_2$H; R$_3$ = R$_4$ = H:
 PTP1B IC$_{50}$ = 2.19 µM
c) X = O; R$_1$ = butyl; R$_2$ = CH(Bn)CO$_2$H; R$_3$ = R$_4$ = H:
 PTP1B IC$_{50}$ = 0.44 µM
d) X = O; R$_1$ = Bn; R$_2$ = CH(Bn)CO$_2$H; R$_3$ = R$_4$ = H:
 PTP1B IC$_{50}$ = 0.32 µM

27% decrease in plasma glucose in ob/ob mouse at 25 mg/kg/day p.o.
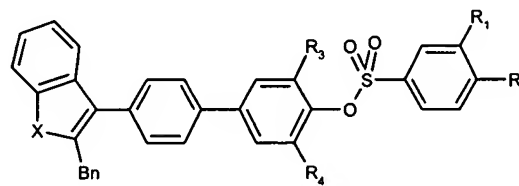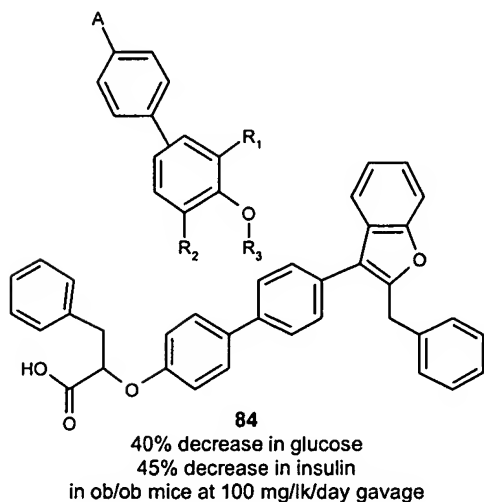e) X = S; R$_1$ = Bn; R$_2$ = CH(Bn)CO$_2$H; R$_3$ = R$_4$ = H:
 PTP1B IC$_{50}$ = 0.095 µM
f) X = S; R$_1$ = Bn; R$_2$ = CH(Bn)CO$_2$H; R$_3$ = R$_4$ = Br:
 PTP1B IC$_{50}$ = 0.025 µM
g) X = S; R$_1$ = Bn; R$_2$ = CH$_2$CO$_2$H; R$_3$ = R$_4$ = 4-MeO-Ph:
 PTP1B IC$_{50}$ = 0.025 µM

84
40% decrease in glucose
45% decrease in insulin
in ob/ob mice at 100 mg/lk/day gavage

85
R$_1$ = OH; R$_2$ = CO$_2$H; R$_3$ = R$_4$ = H:
PTP1B IC$_{50}$ = 0.028 µM

between PPARγ activity and *in vivo* antihyperglycemic potency. The activity of the azolidinediones for PPARγ was not disclosed [178].

A variety of other small molecule PTP inhibitors have been patented; presumably these have arisen from screening programmes. An American Home Products patent is based on substituted aminothiazole acetic acids, with long chain unsaturated substituents, such as in compound **93**, giving

IC$_{50}$ = 0.267 µM versus PTP1B [349]. No *in vivo* data were presented, indicating that these derivatives would probably have poor solubility. Sugen obtained a number of closely related patents describing bilobal thiazole based compounds joined by a single atom linker to a second heteroaryl derivative **(94)**, with the best results given by triazole and tetrazole rings [350-352]. These derivatives showed phosphatase inhibitory activity in an ELISA assay measuring dephos-

86
25% decrease in glucose
in ob/ob mice at 25 mg/lk/day gavage

87
PTP1B IC_{50} = 0.130 µM

88
PTP1B IC_{50} = 0.193 µM

89
PTP1B IC_{50} = 0.015 µM

90
40% decrease in glucose
in ob/ob mice at 100 mg/lk/day gavage

91
a) R = Et: PTP1B 66% inhibition at 2.5 µM
b) R = H: PTP1B IC_{50} = 0.354 µM

92
a) R_1 = CH_3; n = 3; R_2 = Me; R_3 = H
rPTP1B IC_{50} = 1.9 µM; 58% decrease in plasma glucose
86% decrease in plasma insulin; in ob/ob mouse at 100 mg/kg/day
b) R_1 = CH_3; n = 3; R_2 = CH_3; R_3 = H; hPTP1B IC_{50} = 0.3 µM
25% decrease in plasma glucose; 30% decrease in plasma insulin
in ob/ob mouse at 100 mg/kg/day c) R_1 = CH_3; n = 1; R_2 = (Z)- nOct
R_3 = CH_2CO_2H; hPTP1B IC_{50} = 0.12 µM, inactive *in vivo*

93
PTP1B IC$_{50}$ = 0.267 µM

94
X = S,O,NR$_5$, CR$_5$R$_6$
A = heteroaryl (e.g. tetrazole, triazole)

95
inhibition of dephosphorylation of
insulin receptor by ELISA assay:
EC$_{50}$ = 7.8 µM

96
Inhibition of dephosphorylation of
insulin receptor by ELISA assay:
EC$_{50}$ = 3.9 µM

97
a) R$_1$ - H; R$_2$ = Ph:
no biological data
b) R$_1$ = H; R$_2$ = tBu
inhibition of dephosphorylation of
insulin receptor by ELISA assay:
EC$_{50}$ = 4.1 µM

98
PTP1B: IC$_{50}$ = 2.5 µM
SHP2: IC$_{50}$ = 1.8 µM
PTPε: IC$_{50}$ = 8.4 µM
PTPMEG2: IC$_{50}$ = 12.9 µM
PTPσ: IC$_{50}$ = 20 µM
PTPβ: IC$_{50}$ = 6.4 µM
PTPµ: IC$_{50}$ = 6.7 µM

phorylation of the insulin receptor in NIH3T3 cells, with EC$_{50}$ values in the low micromolar range (7.8 µM for compound **95** [350], 3.9 µM for compound **96** [350], 4.1 µM for compound **97**[352]). A nitro group on the thiophene ring appears important for activity and may be binding in the active site. Sugen also claimed formulations containing nitrothiazoles [353]. A Sugen patent claimed phosphate mimics containing two phenyl groups joined by a linker with at least one phenyl substituted with a trifluoromethyl-sulfone or trifluoromethylsulphonamide, such as compound **98**. These acted as nonspecific inhibitors of a number of PTPs (IC$_{50}$ SHP, PTP1B, PTP-ε, MEG2, PTP-σ, PTP-β, PTP-µ = 1.8, 2.5, 8.4, 12.9, 20, 6.4, 6.7 µM, respectively). Indoles with the trifluorosulphonamide substituent were also claimed [354].
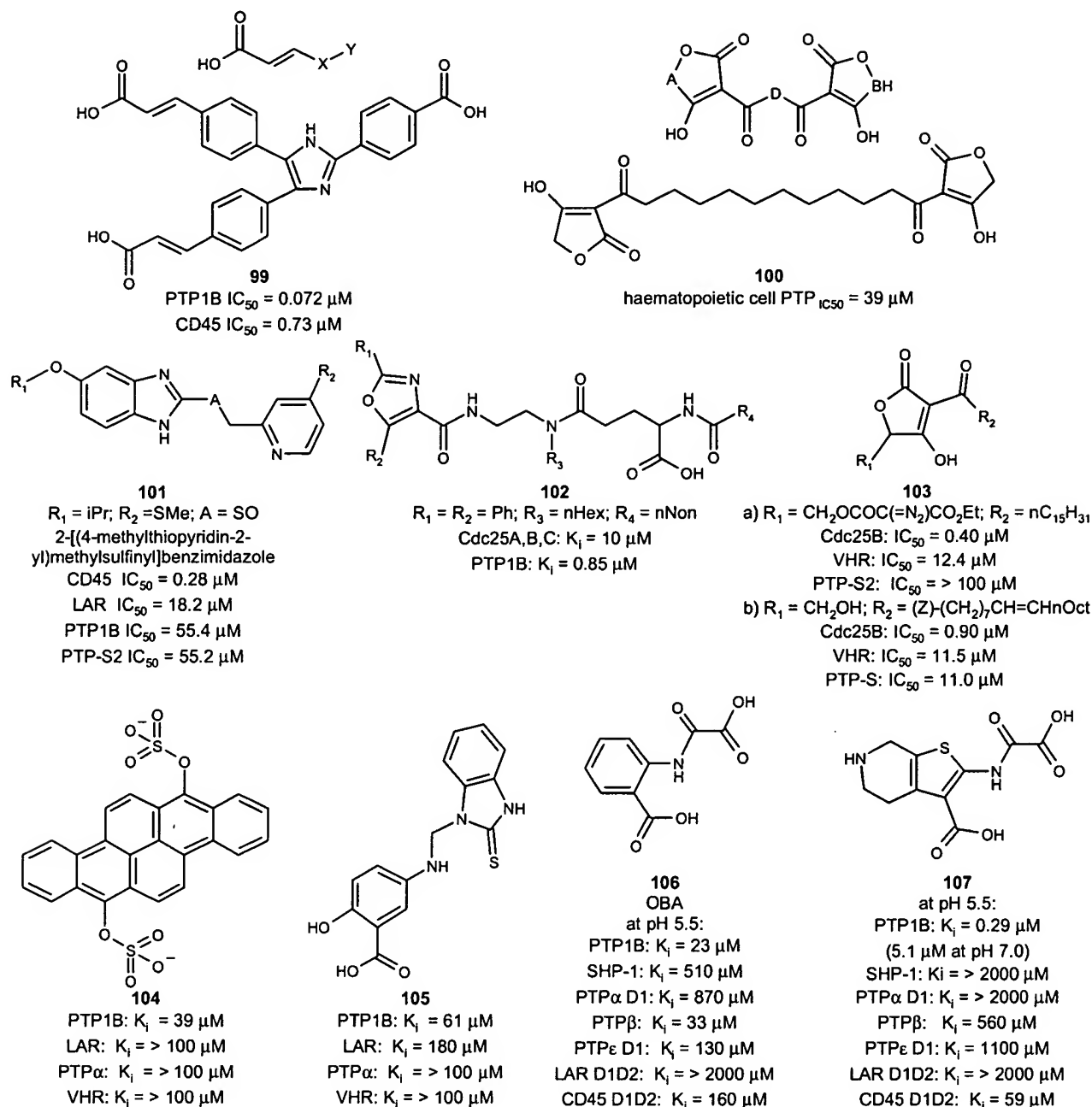
Ontogen has patented > 100 compounds based on multicylic carboxylic acids with an acrylic acid moiety (**99**), with the most potent compound being an imidazole-linked triacid with two cinnamic acid groups, showing IC$_{50}$ = 72 nM for PTP1B and 10-fold selectivity over CD45 (IC$_{50}$ = 0.73 µM) [355]. Alkyl-linked bis(lactone) derivatives were patented by Toray KK, with the best compound (**100**) possessing IC$_{50}$ = 39 µM against a haematopoietic cell PTP [356]. A series of benzimidazoles (**101**) were described by a Japanese group connected with Taisho Pharmaceutical, with the preferred compound showing IC$_{50}$ = 0.28 µM for CD45, with some selectivity over other PTPs [132].

A small library of 18 compounds based on a core template (**102**) designed from natural product PTP inhibitors provided several competitive inhibitors of the DSP Cdc25 that also showed non-competitive inhibition of PTP1B (K$_i$ = 10 µM and 0.85 µM, respectively, for the preferred compound) [179]. A Japanese collaboration produced a library of tetronic acids (**103**). Compounds selective for the DSP cdc25B (IC$_{50}$ = 0.4 µM, vs 12 µM for VHR) were identified; less active PTP-S2 inhibitors (IC$_{50}$ = 11 µM) were also found [180].
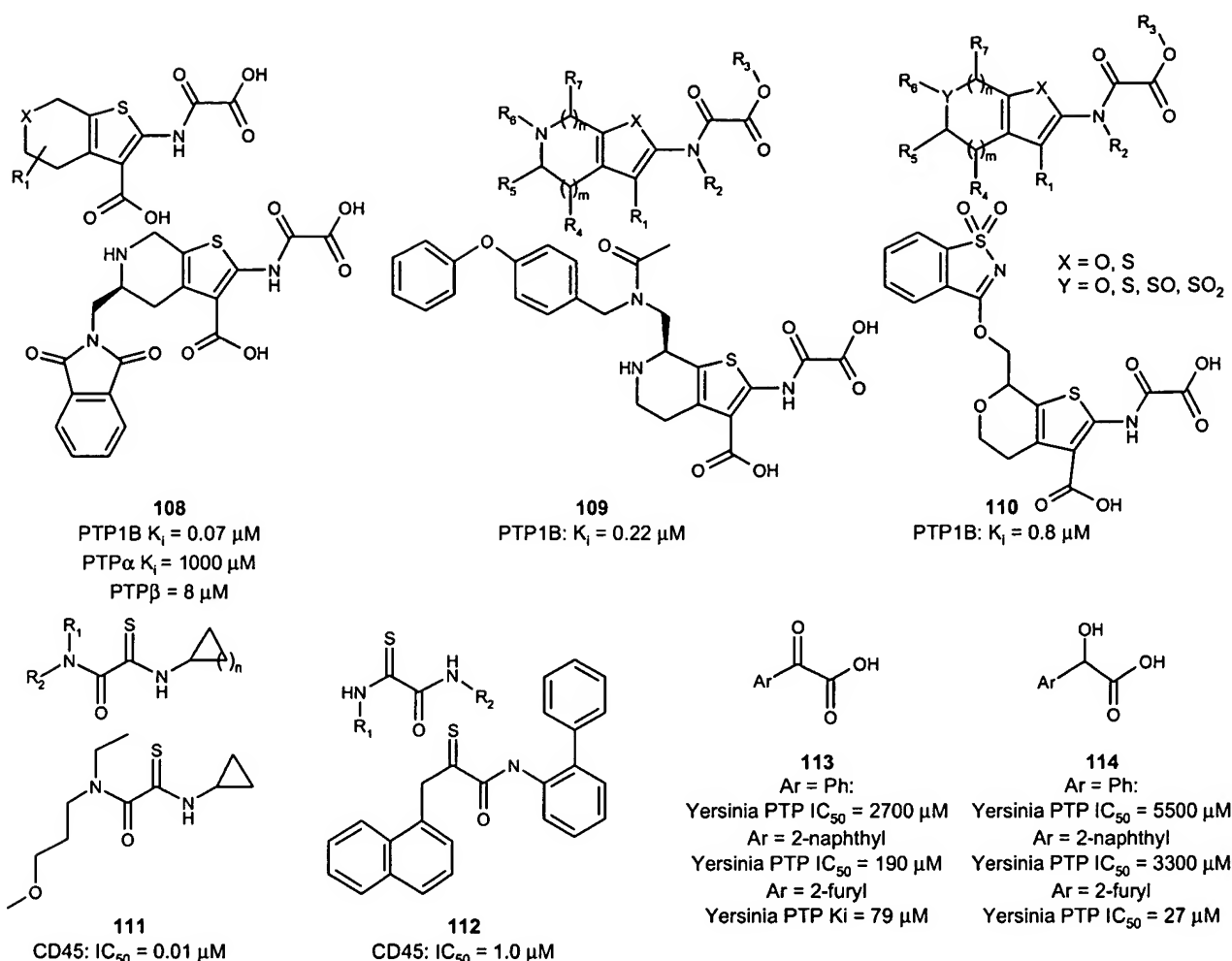
2.5 Rational design of PTP inhibitors

The availability of a variety of X-ray crystal structures of PTPs (in particular PTP1B) bound with a range of inhibitors has provided the opportunity for rational, structure-based design of better inhibitors. For example, a structure-based computer-assisted search of the available chemicals directory (ACD) database using the DOCK progam identified a number of structurally diverse inhibitors, such as compounds 104 and 105, that were selective for PTP1B with K$_i$ = 21 – 510 µM [181].

A group from Novo Nordisk/Ontogen employed structure-based design, using PTP mutants and X-ray protein crystallography, to obtain selective small molecule PTP1B inhibitors [79]. The starting point was 2-(oxalyl-amino)benzoic acid (OBA) (**106**), a general competitive inhibitor of PTPs with PTP1B IC$_{50}$ = 23 µM at pH 5.5 [62] that was identified from high-throughput screening. Crystal structures of PTP1B cocrystallised with OBA and three related analogues show that the

99
PTP1B IC$_{50}$ = 0.072 µM
CD45 IC$_{50}$ = 0.73 µM

100
haematopoietic cell PTP $_{IC50}$ = 39 µM

101
R$_1$ = iPr; R$_2$ =SMe; A = SO
2-[(4-methylthiopyridin-2-
yl)methylsulfinyl]benzimidazole
CD45 IC$_{50}$ = 0.28 µM
LAR IC$_{50}$ = 18.2 µM
PTP1B IC$_{50}$ = 55.4 µM
PTP-S2 IC$_{50}$ = 55.2 µM

102
R$_1$ = R$_2$ = Ph; R$_3$ = nHex; R$_4$ = nNon
Cdc25A,B,C: K$_i$ = 10 µM
PTP1B: K$_i$ = 0.85 µM

103
a) R$_1$ = CH$_2$OCOC(=N$_2$)CO$_2$Et; R$_2$ = nC$_{15}$H$_{31}$
Cdc25B: IC$_{50}$ = 0.40 µM
VHR: IC$_{50}$ = 12.4 µM
PTP-S2: IC$_{50}$ = > 100 µM
b) R$_1$ = CH$_2$OH; R$_2$ = (Z)-(CH$_2$)$_7$CH=CHnOct
Cdc25B: IC$_{50}$ = 0.90 µM
VHR: IC$_{50}$ = 11.5 µM
PTP-S: IC$_{50}$ = 11.0 µM

104
PTP1B: K$_i$ = 39 µM
LAR: K$_i$ = > 100 µM
PTPα: K$_i$ = > 100 µM
VHR: K$_i$ = > 100 µM

105
PTP1B: K$_i$ = 61 µM
LAR: K$_i$ = 180 µM
PTPα: K$_i$ = > 100 µM
VHR: K$_i$ = > 100 µM

106
OBA
at pH 5.5:
PTP1B: K$_i$ = 23 µM
SHP-1: K$_i$ = 510 µM
PTPα D1: K$_i$ = 870 µM
PTPβ: K$_i$ = 33 µM
PTPε D1: K$_i$ = 130 µM
LAR D1D2: K$_i$ = > 2000 µM
CD45 D1D2: K$_i$ = 160 µM

107
at pH 5.5:
PTP1B: K$_i$ = 0.29 µM
(5.1 µM at pH 7.0)
SHP-1: Ki = > 2000 µM
PTPα D1: K$_i$ = > 2000 µM
PTPβ: K$_i$ = 560 µM
PTPε D1: K$_i$ = 1100 µM
LAR D1D2: K$_i$ = > 2000 µM
CD45 D1D2: K$_i$ = 59 µM

inhibitor binds in the phosphate binding loop with a binding mode similar to that of the natural substrate, but with some additional novel binding interactions. This non-selective inhibitor was redesigned via introduction of a fused piperidine ring, so that it could interact with Asp48, a residue that is unique to the PTP1B family of PTPs, resulting in a combination of electrostatic attractions for PTP1B and electrostatic repulsions against undesired PTPs [182]. The modifications resulted in compound 108, with PTP1B K$_i$ = 0.29 µM at pH 5.5 and greatly improved selectivity (> 60-fold) over six other PTPs. At pH 7.0 the activity of compound 107 was reduced (K$_i$ = 5.1 µM) but selectivity was maintained. A crystal structure of
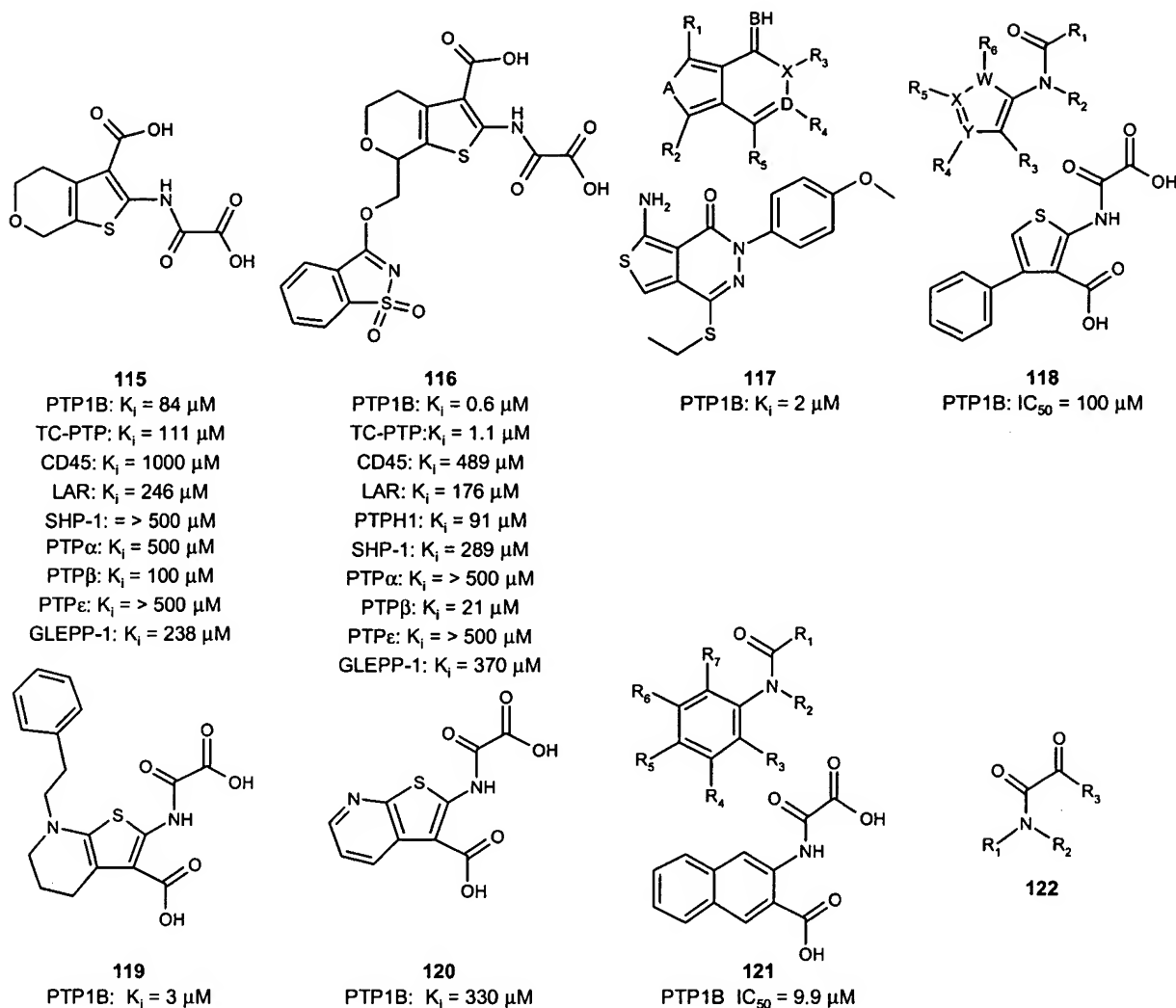
PTP1B with compound 107 shows that a salt bridge is possible [182]. These novel compounds were disclosed in three patents: one specifically claiming compounds inhibiting PTPs with a PTP1B Asp48 type residue, (with compound 108 possessing PTP1B K$_i$ = 0.07 µM, PTP-α = 1000 µM, PTP-β = 8 µM) [358], another more general patent claimed the class of oxalylamino derivatives with a 4,5,6,7-tetrahydrothieno- or -furo[2,3-c]pyridine core (109) as PTP inhibitors (PTP1B K$_i$ = 0.22 µM) [359], while the third patent claimed compounds with heteroatoms other than nitrogen in the fused ring, such as compound 110, which had reduced activity against PTPs with an Asp48 equivalent [360].

X = O, S
Y = O, S, SO, SO₂

108
PTP1B K_i = 0.07 µM
PTPα K_i = 1000 µM
PTPβ = 8 µM

109
PTP1B: K_i = 0.22 µM

110
PTP1B: K_i = 0.8 µM

111
CD45: IC_{50} = 0.01 µM

112
CD45: IC_{50} = 1.0 µM

113
Ar = Ph:
Yersinia PTP IC_{50} = 2700 µM
Ar = 2-naphthyl
Yersinia PTP IC_{50} = 190 µM
Ar = 2-furyl
Yersinia PTP Ki = 79 µM

114
Ar = Ph:
Yersinia PTP IC_{50} = 5500 µM
Ar = 2-naphthyl
Yersinia PTP IC_{50} = 3300 µM
Ar = 2-furyl
Yersinia PTP IC_{50} = 27 µM

The oxalylamino diketo-functionality of OBA has also been shown in PTP inhibitor compounds from other research groups. Two patents by Taisho Pharmaceutical cover N-cycloalkyl thiooxamide derivatives containing a pharmacophore very similar to the Novo Nordisk/Ontogen oxaylamide, such as compound 111, which is a surprisingly potent CD45 inhibitor (IC_{50} = 0.01 µM) [361] and a more general Japanese patent for N-arylthioxamide derivatives, such as compound 112 (CD45 IC_{50} = 1.0 µM) [362]. The compounds are claimed to inhibit activation of mast cells and T cells that participate in immune disorders. Aryl α-ketocarboxylic acids (113) have also been identified as PTP inhibitors, with furyl-2-(α-ketoacetic acid) having K_i = 79 µM against *Yersinia* PTP. The corresponding α-hydroxy derivative 114 had IC_{50} = 27 µM [183].

A second structure-based approach from the Novo Nordisk/Ontogen research group used steric fit and hindrance, rather than electrostatic effects, to obtain PTP1B selective compounds 115 and 116, making use of Gly259 in PTP1B to fit substrate substituents that are too big to be accommodated by other PTPs with a bulky residue at the same position [67]. The preferred compound (116) had PTP1B K_i = 0.6 µM, with > 100-fold selectivity against most other PTPs

(except TC-PTP); a crystal structure of bound inhibitor was again obtained [67]. A patent was filed for bicyclic heteroaryl compounds, in particular those with an aminothiophene moiety, such as compound 117, which possessed PTP1B IC_{50} = 2 µM [363]. Another patent described oxalyl-substituted compounds; including oxalyl-substituted amino thiazole and related acylated aminoheteroaryl compounds (furanyl, thiophenyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl etc.), such as compound 118, with PTP1B IC_{50} = 100 µM [364]. Other patents claim similar compounds with bicyclic heteroaromatic cores (indolyl, benzo[b]thiophenyl, benzo[b]furanyl, indazolyl etc.) such as compound 119 (PTP1B K_i = 3 µM) [365], compounds with heteroaromatic cores and a fused pyridyl ring (furo[2,3-b]pyridyl, thieno[2,3b]pyridyl, pyrrolo[2,3-b]pyridyl or furo[2,3-c]pyridyl etc.), such as compound 120 (PTP1B K_i = 330 µM) [366] or compounds with polycyclic aromatic cores (amino- phenyl, biphenyl, indenyl or fluorenyl-like cores etc.) such as compound 121 (PTP1B IC_{50} = 9.9 µM) [367]. Compounds based on an oxalylamide moiety (122) with MW < 2500 D that inhibit or modulate PTPs or proteins with SH2 domains have also been claimed [368].

115
PTP1B: K_i = 84 µM
TC-PTP: K_i = 111 µM
CD45: K_i = 1000 µM
LAR: K_i = 246 µM
SHP-1: = > 500 µM
PTPα: K_i = 500 µM
PTPβ: K_i = 100 µM
PTPε: K_i = > 500 µM
GLEPP-1: K_i = 238 µM

116
PTP1B: K_i = 0.6 µM
TC-PTP: K_i = 1.1 µM
CD45: K_i = 489 µM
LAR: K_i = 176 µM
PTPH1: K_i = 91 µM
SHP-1: K_i = 289 µM
PTPα: K_i = > 500 µM
PTPβ: K_i = 21 µM
PTPε: K_i = > 500 µM
GLEPP-1: K_i = 370 µM

117
PTP1B: K_i = 2 µM

118
PTP1B: IC_{50} = 100 µM

119
PTP1B: K_i = 3 µM

120
PTP1B: K_i = 330 µM

121
PTP1B IC_{50} = 9.9 µM

122

2.6 Mechanism-based PTP inhibitors

Several different types of mechanism-based inhibitors of PTPs have been discovered, both by screening programmes and by rational design. The nucleophilicity of the PTP active site cysteine residue thiol (Scheme 1) can be utilised to develop irreversible inhibitors. Ham *et al.* at Chung-Ang University in Korea initially found that protein phosphatases could be inactivated by 2-methyl-1,4-naphthoquinone (**123a**) (menadione) via modification of the active site (cdc25; almost complete inhibition within 5 min at > 10 µM) [184]. In order to improve physicochemical properties, polar groups were added to the napthoquinone (**123**), with the best results provided by a dichlorodihydroxy derivative (**123e**) (nearly 100% inactivation of CD45 at 5 µM and significant inhibition of cell development at the G_1/S phase) [185]. A sulfone analogue (**124**) was also found to selectively inactivate PTP1B with K_i = 3.5 ± 0.5 µM, but no inactivation of LAR, *Yersinia* PTP or cdc25a,b or c at ≤ 40 µM [186]. These compounds are believed to act as Michael acceptors for the active site thiol (Scheme 2).

A similar class of compounds, quinolinediones, were identified from the National Cancer Institute Diversity Set (1990 compounds). Thirty quinolinediones were then evaluated for inhibition of the dual specificity phosphatase Cdc25. The most potent, NSC 663284 (**125**), possessed IC_{50} = 0.21, 4.0 and > 100 µM versus Cdc25, VHR and PTP1B respectively and K_i = 29, 95 and 89 nM versus Cdc25A, Cdc25B$_2$ and Cdc25C, respectively [187]. Other analogues were then synthesised, with no improvement in Cdc25B$_2$ activity but better selectivity for Cdc25 over VHR.

α-Haloacetophenone derivatives (**126**) are photoreversible covalent inhibitors (Scheme 3). The best example irreversibly inhibited PTP1B, SHP-1 and VHR with K_i = 42, 530 and 8900 µM, respectively. Irradiation of the inactivated PTPs restored 30 – 80% of their original phosphatase activity [188]. α-Bromobenzylphosphonate (**127**), an irreversible analogue of the α-hydroxybenzylphosphonates (**17**) described earlier, inactivates *Yersinia* PTP with 50% inactivation in 30 min at 1500 µM. The bromide is quite stable in solution with nucleophiles such as cysteine [189].
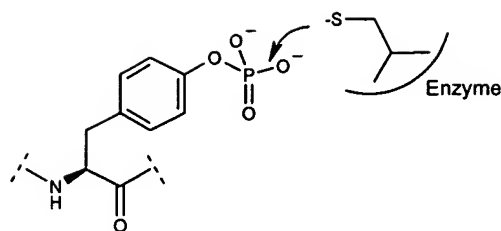
Scheme 1.
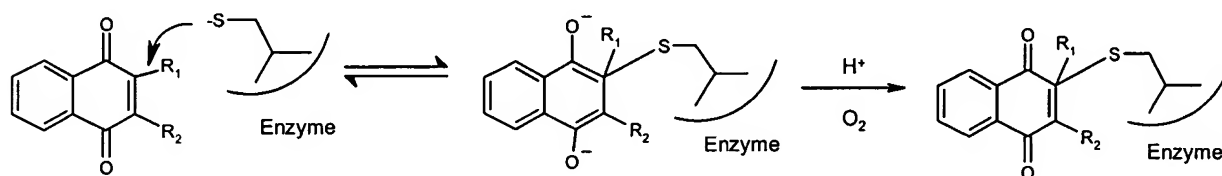
Scheme 2.
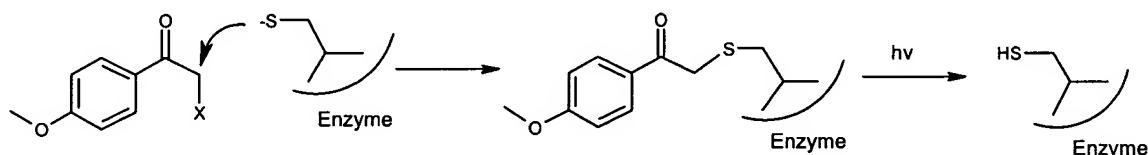
**Scheme 3.**

Dephostatin (128) is an irreversible PTP inhibitor (CD45 IC_{50} = 7.7 µM, [199]) isolated from the culture filtrate of a *Streptomyces* species [190,191,199]. The N-nitrosamine appears to inactivate the active site, with nearly complete inactivation of *Yersinia* PTP within 15 mins at 2800 µM concentration [191]. A series of regioisomeric analogues were prepared, with the 3,4-dihydroxy-N-methyl-N-nitrosoaniline isomer showing equivalent inhibitory activity and improved stability [192]. Further structure–activity relationship studies, along with modelling of dephostatin bound to PTP1B, allowed for the design of an analogue with a methoxime replacing the nitroso group (129) (PTP1B IC_{50} = 2.9 µg/ml) [193].

4-Difluoromethylphenyl bis(cyclohexylammonium) phosphate (130, Scheme 4) is an irreversible inhibitor of human prostatic acid phosphatase (K_i = 1000 µM) and SHP-1 ($t_{1/2}$ = 15 min at 8200 µM concentration) that probably acts via enzymatic release of a difluoromethyl phenol reactive intermediate. Elimination of fluoride from the intermediate generates an electrophilic quinone methide (Scheme 4) [194]. 4-(Fluoromethyl)phenyl phosphate (131) was separately reported to be a mechanism-based inactivator of prostatic acid phosphatase with K_i = 150 µM, again via a putative quinone methide [195]. The monofluoro compound

inactivates 8-fold faster and binds 7-fold tighter than the difluoro compound, apparently due to greater stability of the difluoromethylphenol intermediate slowing decomposition to the quinone methide. All of these inhibitors would potentially be quite toxic *in vivo*.

A group from AstraZeneca employed high-throughput screening to identify 9,10-phenanthrenediones (132) as lead compounds for inhibition of cytosolic CD45 (best IC_{50} = 3 µM). The 1,2-dione moiety is a critical feature that is hypothesised to act as an electrophilic centre that reacts with the active site thiol [196]. A series of analogues were prepared, including attachment to a peptide sequence. The most potent derivative (132) had CD45 IC_{50} = 0.2 µM, selectivity for CD45 over PTP1B and inhibited T cell receptor-mediated proliferation with low micromolar activity [196]. A series of patents claimed for CD45 inhibitors for use in treating immunological-related diseases, with one claiming compositions containing tricyclic heteroaryl compounds, such as 133 (CD45 IC_{50} = 1.0 µM, T cell proliferation IC_{50} = 0.24 µM) [369], another for the napthalenedione core structure such as (134) (CD45 IC_{50} = 1.2 or 7.7 µM) [370] and a third for phen-antheren-9,10-diones (135), which appear to be the most active class (for specified compound CD45 IC_{50} = 0.2 µM,
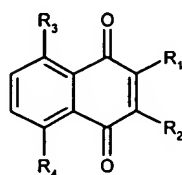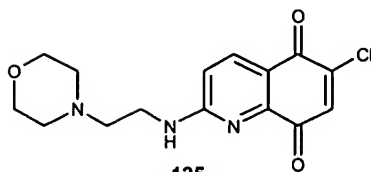
123

a) $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$: menadione
cdc25: > 90% inhibition in 15 min at 10 μM
b) $R_1 = H$, CH_3, NH_2, NHR, OH, SH, OCH_2CH_3, SCH_2CH_3;
$R_2 = R_3 = R_4 = H$
c) $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = OH$
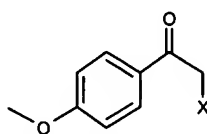d) $R_1 = R_2 = H$; $R_3 = R_4 = OH$
e) $R_1 = R_2 = Cl$; $R_3 = R_4 = OH$ (active vs Cdc25A)

124
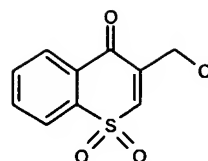
PTP1B: $K_i = 3.5$ μM
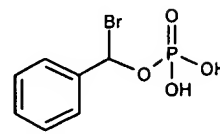no inhibition at 40 μM for
cdc25A, cdc25B, cdc25C,
LAR and Yersinia PTP

125
NSC 663284
Cdc25: $IC_{50} = 0.21$ μM
VHR: $IC_{50} = 40$ μM
PTP1B: $IC_{50} = > 100$ μM

126
R = H, CH_3, CH_2CO_2H; X = Br, Cl
for R = H, X = Br
PTP1B: $K_i = 42$ μM
SHP-1: $K_i = 530$ μM
VHR: $K_i = 8900$ μM

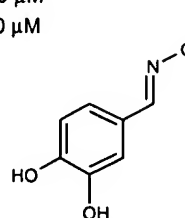**127**
Yersinia PTP: 50% inactivation
in 30 min at 1500 μM
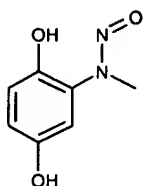
128
Dephostatin
CD45: $IC_{50} = 7.7$ μM

129
Dephostatin analogue
PTP1B: $IC_{50} = 2.9$ μg/mL

T cell proliferation $IC_{50} = 0.1$ μM [371]. A Japanese group has isolated nocardione A (136), produced by *Nocardia* sp. TP-A0248, which inhibits Cdc25B ($IC_{50} = 17$ μM), PTP1B ($IC_{50} = 14$ μM) and FAP-1 ($IC_{50} = 89$ μM) and shows antifungal/cytotoxic activity [197]. It has been patented for treatment of tumours and fungal infections via inhibition of Cdc25B [372]. The napthylfuranone possesses the diketo moiety found in the AstraZeneca 9,10-phenanthrenediones.

Succinic acid (137) has been patented as a PTP inhibitor that acts via enhancement of endogenous hydrogen peroxide that causes reversible inactivation of PTP. Administration of 200 mg/day to noninsulin dependent diabetics allowed for a 50% reduction in insulin dose over 60 days. Wound healing in rats was also enhanced via topical application [373].

2.7 Other approaches to PTP inhibition

Other methods can be used that result in the same biochemical effect as direct inhibition of PTPs. An unusual alternative approach is to trap PTP substrates using mutant PTPs. A patent for the Asp181Ala PTP1B mutant claims that if this mutant protein is administered to a human it would be able to trap the substrate and inhibit the signalling pathway,

providing a potentially useful treatment for cancer [301]. Another approach that gives the same result as PTP inhibition is to inhibit expression of the PTP. ISIS Pharmaceuticals has patented antisense chimeric oligonucleotide compounds that inhibit the expression of human PTP1B [374], SHP-1 [375] and SHP-2 [376]. Ribozyme has patented enzymatic nucleic acid polymers, antisense molecules and triplex DNA for inhibiting expression of genes affecting the development and progression of cancers, diabetes, obesity and other diseases, in particular the gene for PTP1B [377]. Other approaches that have been patented include regulation of RPTP-β/ζ by the cytokine pleiotropin [378] and the use of antibodies against receptor-type PTPs for both treatment and diagnosis indications [379].

3. Expert opinion

The past five years have seen an increased understanding of the pivotal role PTPs play in many physiological processes, a realisation that has translated into a significant research effort towards the development of drug-like PTP inhibitors. The greatest activity has been devoted towards identifying

130
SHP-1: $t_{1/2}$ = 15 min at 8200 μM
human prostatic acid
phosphatase K_i = 1000 μM
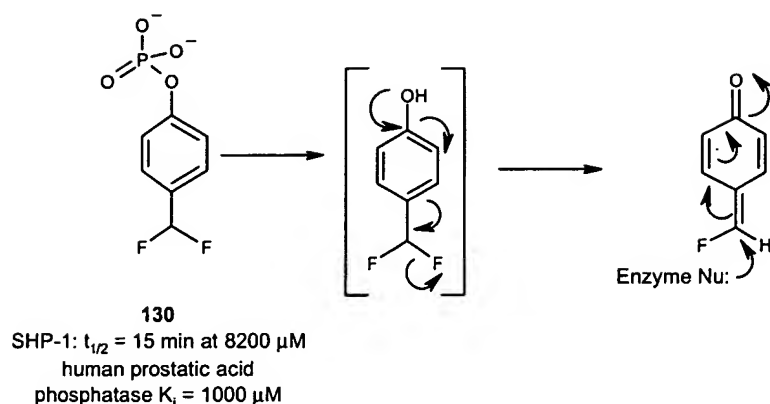
Scheme 4.

potent and selective inhibitors of PTP1B, as there is strong evidence that PTP1B is a valid target with a significant therapeutic role. The primary disease states associated with PTP1B, Type II diabetes and obesity have enormous market potential. Most patented and published research on PTP1B inhibitors has come from groups at Merck Frosst Canada, Wyeth-Ayerst/American Home Products and Novo-Nordisk/Ontogen, along with contributions from Sugen, AstraZeneca and Pharmacia & Upjohn. Wyeth-Ayerst appears to have the only PTP inhibitor that has passed the discovery stage and into clinical trials; as of January 2002 (138), from the benzbromarone-derived series of compounds, was in Phase II clinical trials for non-insulin-dependent diabetes. An organovanadium PTP inhibitor developed by Kinetek Pharmaceuticals, KP-100, was scheduled to enter Phase I trials in early 2000 but was discontinued. Merck/ISIS have an antisense inhibitor of the PTP1B gene as a potential Phase I clinical candidate.

Research into the inhibition of other PTP targets is less advanced but will undoubtedly increase in the near future as the roles of other PTPs in signalling events is untangled. Both AstraZeneca and Taisho are examining CD45 inhibitors for the treatment of immune-related diseases, while inhibitors of cdc25 are showing potential as anticancer agents. However, obtaining unequivocal evidence of the consequences of inhibiting individual PTPs is not trivial, especially if PTP-knockout animal models are not viable or suffer from severe defects. An additional complication is the possible role of individual PTPs in multiple signalling pathways, leading to undesirable side effects. The validation of other PTPs as therapeutic targets will be difficult.

A significant challenge facing future development of PTP inhibitors will be to obtain compounds selective for the desired PTP. This obstacle appears to have been overcome for PTP1B compared with structurally different PTPs, such as CD45, but selectivity against closely related TCPTP is generally glossed over or ignored in most reports. It remains to be seen whether selective inhibition within families of other

closely related PTPs, such as the receptor PTPs, is possible to attain. The use of structural information, in particular X-ray crystal structures, will play an important role in identifying differences between PTPs that can be utilised to design selective inhibitors. An additional obstacle for future therapeutic drug development is the conversion of lead compounds derived from peptides or pTyr-mimetics into more drug-like entities, paying attention to critical ADME properties such as metabolic stability and oral availability. The phosphonate moiety (and related isosteres) that is generally essential for potent PTP inhibition is also responsible for poor cell permeability. Strategies must be developed to overcome this dichotomy in order for PTP inhibitors to have widespread therapeutic application.

Note added in proof

Two more relevant articles have recently appeared. One of them addresses the question of whether a PTPase–vanadate complex is a true transition state analogue, which is a critical consideration if crystal structures of these complexes are being used to design inhibitors. Steady-state kinetic measurements and difference Raman measurements of vanadate bound to *Yersinia* PTP and a number of mutants indicates an associative transition state complex, so that vanadate is not a true transition state analogue [201]. In the other, Pharmacia reports the results of molecular docking of 235,000 commercial compounds and high-throughput screening of a corporate library of 4000,000 compounds for novel inhibitors of PTP1B [202]. The experimental assay identified 85 compounds (0.021%) with IC_{50} < 100 μM, while 127 of 365 compounds suggested by the docking method (34.8%) had similar activity. The two hit lists were structurally dissimilar from each other and from phosphotyrosine, and the docking hits appeared to possess more drug-like properties. The docking hits have some resemblance to at least one of those, compound **105**, identified by another group via a similar method [181].
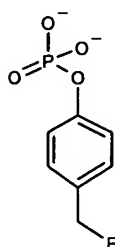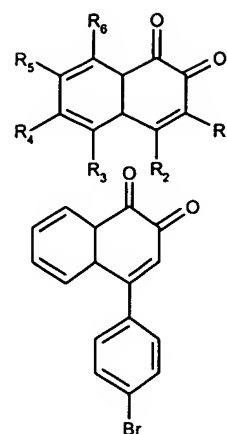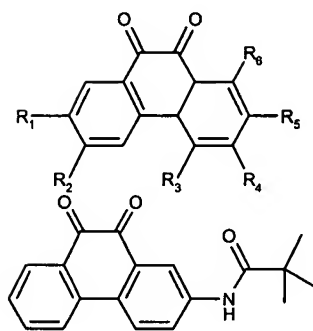
131
human prostatic
acid phosphatase
K_i = 150 µM
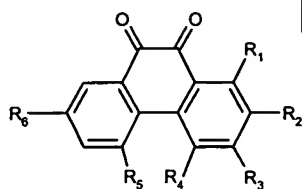
132
R_1 = H; R_2 = NHCOtBu;
R_3 = R_4 = R_5 = R_6 = H
CD45: IC_{50} = 0.2 µM
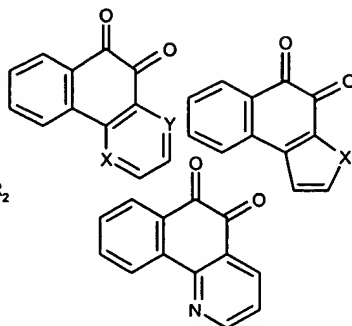
133
CD45: IC_{50} = 1.0 µM
T cell proliferation: IC_{50} = 0.24 µM

134
a) R = OCH_3; CD45: IC_{50} = 1.2 µM
b) R = 4-Br-Ph; CD45: IC_{50} = 7.7 µM
T cell proliferation: IC_{50} = 0.11 µM

135
CD45: IC_{50} = 0.2 µM
T cell proliferation: IC_{50} = 0.1 µM
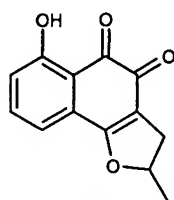
136
nocardione A
PTP1B: IC_{50} = 14 µM
Cdc25B: IC_{50} = 17 µM
FAP-1: IC_{50} = 89 µM
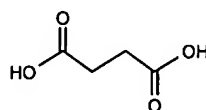
137
succinic acid
200 mg/day caused
50% decrease in
insulin dose for non-insulin
dependent diabetics
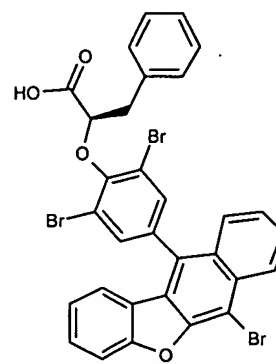
138
Wyeth-Ayerst Research
Phase II

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